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(54) Title: SCAFFOLD-FREE SELF-ORGANIZED 3D SYNTHETIC TISSUE

(57) Abstract: The present invention can be used for actual implantation surgery without a scaffold. The present invention provides a synthetic tissue or complex, which can be produced by culture and has a high level of differentiation ability. The present invention also provides a therapy and medicament for repairing and/or regenerating tissue using replacement and covering. By culturing cells under specific culture conditions such that medium contains an extracellular matrix synthesis promoting agent, the cells are organized and are easily detached from a culture dish. The present invention was achieved by finding such a phenomenon. In addition, the self contraction of the tissue can be regulated by culturing the tissue in a suspended manner. Therefore, it is possible to regulate the three-dimensional shape of the tissue. The present invention also provides a method for producing an implantable synthetic tissue which does not require a plurality of monolayer cell sheets assembled to form a three-dimensionally structured synthetic tissue. The present invention is characterized by richness in adhesion molecules, nonnecessity of additional fixation at an implantation site, and good biological integration.

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DESCRIPTION

SCAFFOLD-FREE SELF-ORGANIZED 3D SYNTHETIC TISSUE

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TECHNICAL FIELD

The present invention relates to the field of regenerative medicine. More particularly, the present invention relates to a synthetic tissue capable of functioning after implantation, a method for producing the same, and use of the same. The synthetic tissue of the present invention has biological integration capability.

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BACKGROUND ART

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Recently, regenerative therapy has attracted attention as a novel approach to severe organ failure or intractable diseases. Regenerative therapy is a combination of genetic engineering, cell tissue engineering, regenerative medicine, and the like. Many researchers over the world are vigorously working on this important and challenging subject of research in the 21-century advanced medical practice.

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The scale of the market associated with regenerative medicine (tissue engineering) is estimated as about 500 billion US dollars in the world and about 50 billion US dollars in Japan according to the material prepared by the New Energy and Industrial Technology Development Organization. Only tissue engineering products account for about 100 billion US dollars in the world. The regenerative medicine is greatly expected to create the next-generation industry.

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The present inventors have made efforts to develop regenerative therapy in the field of musculoskeletal and cardiovascular tissues, and have reported a combination therapy of cell implantation and a growth factor administration, or a tissue implantation regeneration therapy based on tissue engineering. However, regenerative therapy based on cell or tissue implantation requires a source of autologous cells. A stable and abundant source of such cells is urgently required and important. A number of cells in musculoskeletal tissue have a high level of self-repairing ability. It has been reported that there is a stem cell among the cells of the musculoskeletal tissue.

It has been demonstrated that a cell derived from skeletal muscle (Jankowski R.J., Huand J. et al., *Gene Ther.*, 9:642-647, 2002), fat (Wickham M.Q. et al., *Clin. Orthop.*, 2003, 412, 196-212), umbilical cord blood (Lee O.K. et al., *Blood*, 2004, 103:1669-75), tendon (Salingmarnboriboon R., *Exp. Cell. Res.*, 287:289-300, 2002), bone marrow (Pitterger M.F. et al., *Science*, 284:143-147, 1999), and synovium (*Arthritis Rheum.* 2001 44:1928-42) is undifferentiated and has the potential to differentiate into various cells.

Conventionally, when cell therapy is performed for repair or regeneration of tissue, most research employs a biological scaffold to maintain the accumulation of cells, allow cells to grow, maintain pluripotency, protect cells from mechanical stress on a treated site, or the like. However, most scaffolds contain a biological (animal) material, a biomacromolecule material, or the like, of which influence on the safety of organism cannot be fully predicted.

A cell implanting method without a scaffold has been

reported by Kushida A., Yamato M., Konno C., Kikuchi A., Sakurai Y., Okano T., J. Biomed. Mater. Res., 45:355-362, 1999, in which a cell sheet is produced using a temperature sensitive culture dish. Such a cell sheet engineering technique is internationally appraised due to its originality. However, a single sheet obtained by this technique is fragile. In order to obtain the strength that can withstand surgical manipulation, such as implantation, a plurality of sheets need to be assembled, for example.

When a nano-biointerface technology is used to fix a temperature responsive polymer (PIPAAm) onto a plastic mold, such as a Petri dish, for cell culture, the polymer surface is reversibly changed at 31°C between hydrophilicity and hydrophobicity. Specifically, when the temperature is 31°C or more, the surface of the Petri dish is hydrophobic so that cells or the like can adhere thereto. In this situation, the cells secrete extracellular matrix (ECM; for example, adhesion molecules which are proteins having a function like a "glue") and adhere to the surface of the Petri dish, so that the cells can grow. See, Okano T., Yamada N., Sakai H., Sakurai Y., J. Biomed. Mater. Res., 1993, 27:1243-1251; Kushida A., Yamato M., Konno C., Kikuchi A., Sakurai Y., Okano T., J. Biomed. Mater. Res. 45:355-362, 1999; and Shimizu T., Yamato M., Akutsu T. et al., Circ. Res., 2002, Feb 22; 90(3):e40.

When the temperature is 31°C or less, the surface of the Petri dish is hydrophilic. The cells which have adhered to the Petri dish are readily detached, though the cells still maintain adhesion molecules. This is because the surface of the Petri dish to which the cells have adhered no longer exists at 31°C or less.

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Even when such a Petri dish having a fixed temperature responsive polymer (e.g., tradename: UpCell and RepCell) is used to culture cells and detach the cells, an extracellular matrix is not appropriately provided. Thus, there has been no actually practical synthetic tissue developed. See, Okano T., Yamada N., Sakai H., Sakurai Y., J. Biomed. Mater. Res., 1993, 27:1243-1251; Kushida A., Yamato M., Konno C., Kikuchi A., Sakurai Y., Okano T., J. Biomed. Mater. Res. 45:355-362, 1999; and Shimizu T., Yamato M., Akutsu T. et al., Circ. Res., 2002, Feb 22; 90(3):e40.

WO00/51527 and WO03/024463 reported that cells are cultured on a semipermeable membrane using alginate gel. However, the resultant tissue is poorly integrated with an extracellular matrix and is not free of a scaffold. In addition, the cells in the tissue are not self organized. The tissue has no self-supporting ability. The cells no longer have a differentiation potential. The tissue loses morphological plasticity in terms of three-dimensional structure. Therefore, the tissue is not suitable for cell implantation.

Use of a scaffold is considered to be problematic in implantation therapy because of adverse side effects. Therefore, there is a demand for the advent of a scaffold-free technique.

Conventional methods for producing tissue sheets have the following drawbacks: it is not possible to produce a very large sized sheet; it is not possible to produce a sheet having biological integration in three dimensions; when a sheet is detached after sheet production, the sheet

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is broken into pieces; and the like.

Therefore, there is a keen demand for a synthetic tissue, which is developed by culture processes, capable of withstanding an implantation operation, capable of being used in an actual operation.

By conventional techniques, it is difficult to isolate a synthetic tissue from a culture base material after tissue culture, and it is substantially impossible to produce a large sized tissue piece. Therefore, conventional synthetic tissues, such as tissue sheets, cannot be used in medical application in view of size, structure, mechanical strength, and the like. It is difficult to develop a synthetic tissue using conventional techniques. Therefore, unfortunately their supplies are limited.

An object of the present invention is to provide a synthetic tissue produced by cell culture, which is feasible to implantation surgery.

Specifically, an object of the present invention is to provide a synthetic tissue having a three-dimensional structure and self-supporting ability, being free of a scaffold, and maintaining a differentiation potential if the tissue possesses it.

Still another object of the present invention is to provide a method and a pharmaceutical agent for treating an injury of a tissue or the like when a replacement or resurfacing therapy is required.

DISCLOSURE OF THE INVENTION

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The above-described objects were achieved in part based on the invention of the following synthetic tissue. When a cell was cultured in medium containing an extracellular matrix (ECM) synthesis promoting agent, cells and ECM produced by the cells are integrated to form a tissue, which was readily detached from the culture dish.

The above-described objects were achieved by providing a synthetic tissue of the present invention which is free of a scaffold, has self-supporting ability, is easily formed into a three-dimensional structure, has morphological plasticity, has excellent ability to biologically adhere to surroundings, has a differentiation potential, and the like, and finding that the synthetic tissue is effective for a replacement or resurfacing therapy at an injured site.

The present invention also provides a method for producing an implantable synthetic tissue, which has biological integration and does not require assembling layers.

The above-described objects were achieved by finding that the thickness of the synthetic tissue of the present invention can be adjusted to a desired value by regulating a physical or chemical stimulus on the synthetic tissue.

The present inventors realized the formation of a three-dimensional synthetic tissue (cellular therapeutic system) comprising cultured cells (e.g., fat-derived cells, etc.) and material produced by the cells without a scaffold.

The synthetic tissue of the present invention can

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be constructed into various shapes and has a sufficient strength. Therefore, it is easy to surgically manipulate (e.g., implant, etc.) the synthetic tissue of the present invention. According to the present invention, a large
5 quantity (e.g., 10^5 to 10^8) of cells can be securely supplied to a local site by means of tissue implantation.

In the matrix, cell adhesion molecules, such as collagen (e.g., type I, type III), fibronectin, vitronectin,
10 and the like, are present in large amounts. Particularly, the cell adhesion molecules are integrated throughout the matrix.

Therefore, the tissue has excellent ability of
15 biologically adhesion to surroundings of the implanted site. Thus, the synthetic tissue complex biologically adheres to an implanted site tissue very quickly. In addition, by changing culture conditions, the synthetic tissue can be differentiated into a bone or cartilage tissue. The
20 maintenance of a differentiation potential is a feature of the synthetic tissue of the present invention which was first found by the present inventors. The synthetic tissue is effective as a safe and efficient cell therapy system.

25 An object of the present invention is to provide a clinical application of the synthetic tissue regeneration of a joint tissue. The present invention provides the above-described synthetic tissue or a complex of a cell and a component derived from the cell, thereby making it possible
30 to develop therapies for bone regeneration at a conventionally intractable site, in which both periosteum and bone cortex are inflamed; partial thickness cartilage injury which does not bleach the subchondral bone, and injury

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of a meniscus, a tendon, a ligament, an intervertebral disk, cardiac muscle in an avascular area or a poor circulation site.

5 Thus, the present invention provides the following.

1. An implantable synthetic tissue.
2. A synthetic tissue according to item 1, which is
10 biologically organized in the third dimensional direction.
3. A synthetic tissue according to item 1, which has biological integration capability with surroundings.
- 15 4. A synthetic tissue according to item 3, wherein the biological integration capability includes capability to adhere to surrounding cells and/or extracellular matrices.
5. A synthetic tissue according to item 1, which comprises
20 cells.
6. A synthetic tissue according to item 1, which is substantially made of cells and a material derived from the cells.
25
7. A synthetic tissue according to item 1, which is substantially made of cells and an extracellular matrix (ECM) derived from the cells.
- 30 8. A synthetic tissue according to item 7, wherein the extracellular matrix contains at least one selected from the group consisting of collagen I, collagen III, vitronectin and fibronectin.

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9. A synthetic tissue according to item 7, wherein the extracellular matrix contains collagen I, collagen III, vitronectin and fibronectin.
- 5 10. A synthetic tissue according to item 7, wherein the extracellular matrix contains vitronectin.
- 10 11. A synthetic tissue according to item 7, wherein the extracellular matrix contains fibronectin.
12. A synthetic tissue according to item 7, wherein the extracellular matrix contains collagen I and collagen III, the collagen constitutes 5% to 25% of the tissue, and the
15 ratio of the collagen I to the collagen III is between 1:10 and 10:1.
13. A synthetic tissue according to item 7, wherein the extracellular matrix and the cells are integrated together
20 into a three-dimensional structure.
14. A synthetic tissue according to item 7, wherein the extracellular matrix is diffusely distributed in the tissue.
- 25 15. A synthetic tissue according to item 1, wherein an extracellular matrix is diffusely distributed, and the distribution densities of the extracellular matrix in two arbitrary sections of 1 cm² in the tissue have a ratio within a range of about 1:3 to about 3:1.
- 30 16. A synthetic tissue according to item 1, which is heterologous, allogenic, isologous, or autogenous.

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17. A synthetic tissue according to item 1, which is free of scaffolds.
18. A synthetic tissue according to item 1, which is used to implant cells.
19. A synthetic tissue according to item 1, which is large sized.
20. A synthetic tissue according to item 1, which has a volume of at least about 20 mm³.
21. A synthetic tissue according to item 1, which is flexible.
22. A synthetic tissue according to item 1, which is expandable and contractile.
23. A synthetic tissue according to item 1, which can withstand heart pulsation.
24. A synthetic tissue according to item 1, which is biologically organized in all three dimensional directions.
25. A synthetic tissue according to item 24, wherein the biological integration is selected from the group consisting of internal binding of extracellular matrix, electrical integration, and intercellular signal transduction.
26. A synthetic tissue according to item 1, which has a tissue strength which allows the synthetic tissue to be clinically applicable.

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27. A synthetic tissue according to item 26, wherein the strength is a break strength of about 0.02 N to about 2 N.

28. A synthetic tissue according to item 26, wherein the
5 tissue strength is sufficient to provide self-supporting ability.

29. A synthetic tissue according to item 26, wherein the self-supporting ability is characterized in that the
10 synthetic tissue is not substantially broken when the synthetic tissue is picked up using forceps having a tip area of 0.05 to 3.0 mm².

30. A synthetic tissue according to item 28, wherein the
15 self-supporting ability is characterized in that the synthetic tissue is not broken when the synthetic tissue is picked up with a hand.

31. A synthetic tissue according to item 26, wherein the
20 site to which the synthetic tissue is intended to be applied, includes a heart.

32. A synthetic tissue according to item 26, wherein the site to which the synthetic tissue is intended to be applied,
25 includes an intervertebral disk, a meniscus, a cartilage, a bone, a ligament, or a tendon.

33. A synthetic tissue according to item 26, wherein:
the synthetic tissue is a cartilage, an
30 intervertebral disk, a meniscus, a ligament, or a tendon;
and

the synthetic tissue remains attached without an additional fixation procedure, after the synthetic tissue

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is implanted into an injured portion of the intra-articular tissue.

5 34. A method for producing a synthetic tissue, comprising the steps of:

A) providing cells;

B) placing the cells in a container, the container having cell culture medium containing an ECM synthesis promoting agent and having a sufficient base area which can
10 accommodate a synthetic tissue having a desired size;

C) culturing the cells in the container along with the cell culture medium containing the ECM synthesis promoting agent for a period of time sufficient for formation of the synthetic tissue having the desired size; and

15 D) detaching the cells from the container.

35. A method according to item 34, wherein a stimulus for inducing tissue contraction is applied in the detaching step.

20 36. A method according to item 35, wherein the stimulus includes a physical or chemical stimulus.

37. A method according to item 36, wherein the physical stimulus includes shaking of the container, pipetting, or
25 deformation of the container.

38. A method according to item 34, wherein the detaching step includes adding an actin regulatory agent.

30 39. A method according to item 38, wherein the actin regulatory agent includes a chemical substance selected from the group consisting of actin depolymerizing agents and actin polymerizing agents.

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40. A method according to item 39, wherein the actin depolymerizing agent is selected from the group consisting of Slingshot, cofilin, cyclase associated protein (CAP),
5 actin interacting protein 1 (AIP1), actin depolymerizing factor (ADF), destrin, depactin, actophorin, cytochalasin, and NGF (nerve growth factor).
41. A method according to item 39, wherein the actin
10 polymerizing agent is selected from the group consisting of RhoA, mDi, profilin, Rac1, IRSp53, WAVE2, ROCK, LIM kinase, cofilin, cdc42, N-WASP, Arp2/3, Drf3, Mena, lysophosphatidic acid (LPA), insulin, platelet derived growth factor (PDGF) α , PDGF β , chemokine, and transforming growth factor (TGF)
15 β .
42. A method according to item 34, wherein the container is free of scaffolds.
- 20 43. A method according to item 34, wherein the cells are first cultured in monolayer culture.
44. A method according to item 34, wherein the ECM synthesis promoting agent includes TGF β 1, TGF β 3, ascorbic acid,
25 ascorbic acid 2-phosphate, or a derivative or salt thereof.
45. A method according to item 44, wherein the ascorbic acid, ascorbic acid 2-phosphate, or the derivative or salt thereof is present at a concentration of at least 0.1 mM.
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46. A method according to item 44, wherein the TGF β 1 or TGF β 3 is present at a concentration of at least 1 ng/ml.

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47. A method according to item 34, wherein the cells are placed at a concentration of 5×10^4 to 5×10^5 cells per 1 cm^2 , and the ECM synthesis promoting agent is ascorbic acid, ascorbic acid 2-phosphate, or a derivative or salt thereof, and the ascorbic acid, ascorbic acid 2-phosphate, or the derivative or salt thereof is provided at a concentration of at least 0.1 mM.
48. A method according to item 34, further comprising causing the synthetic tissue to detach from the container and self-contract.
49. A method according to item 48, wherein the detaching and self-contraction are achieved by providing a physical stimulus to the container.
50. A method according to item 48, wherein the detachment and self-contraction are achieved by providing a chemical stimulus to the container.
51. A method according to item 34, wherein the sufficient period of time is at least 3 days.
52. A method according to item 34, wherein the sufficient period of time is at least 3 days and a period of time required for the synthetic tissue to be spontaneously detached from the container at a maximum.
53. A method according to item 52, wherein the period of time required for the synthetic tissue to be spontaneously detached from the container is at least 40 days.
54. A method according to item 34, further comprising:

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causing the synthetic tissue to differentiate.

55. A method according to item 54, wherein the differentiation includes osteogenesis, chondrogenesis, adipogenesis, tendon differentiation, and ligament differentiation.

56. A method according to item 55, wherein the osteogenesis is performed in medium containing dexamethasone, β -glycerophosphate, and ascorbic acid 2-phosphate.

57. A method according to item 56, wherein the medium contains at least one selected from the group consisting of BMP (bone morphogenetic protein)-2, BMP-4, and BMP-7.

58. A method according to item 55, wherein the chondrogenesis is performed in medium containing pyruvic acid, dexamethasone, ascorbic acid 2-phosphate, insulin, transferrin, and selenious acid.

59. A method according to item 58, wherein the medium contains at least one selected from the group consisting of BMP-2, BMP-4, BMP-7, TGF(transforming growth factor)- β 1 and TGF- β 3.

60. A method according to item 54, wherein the differentiation step is performed before or after the detaching step.

61. A method according to item 54, wherein the differentiation step is performed after the detaching step.

62. A method according to item 34, wherein the cell includes

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cells of 3 or more passages.

63. A method according to item 34, wherein the cells include cells of 3 to 8 passages.

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64. A method according to item 34, wherein the cells are provided at a cell density of 5.0×10^4 to 5.0×10^6 cells/cm².

10

65. A method according to item 34, wherein the cells include myoblasts.

66. A method according to item 34, wherein the cells include fat-derived cells.

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67. A method according to item 34, wherein the cells include synovium-derived cells.

68. A method according to item 34, wherein the cells include mesenchymal stem cells.

20

69. A method according to item 68, wherein the mesenchymal stem cells are derived from an adipose tissue, a synovial membrane, a tendon, a bone, or a bone marrow.

25

70. A method according to item 34, further comprising:
producing a plurality of the synthetic tissues and
attaching the plurality of the synthetic tissues together
to be integrated.

30

71. A cell culture composition for producing a synthetic tissue from cells, comprising:

- A) an element for maintaining the cells; and
- B) an extracellular matrix synthesis promoting

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agent.

72. A method according to item 68, wherein the ECM synthesis
promoting agent includes TGF β 1, TGF β 3, ascorbic acid,
5 ascorbic acid 2-phosphate, or a derivative or salt thereof.

73. A method according to item 72, wherein TGF β 1 or TGF β 3
is present at a concentration of at least 1 ng/ml, or ascorbic
acid, ascorbic acid 2-phosphate, or the derivative or salt
10 thereof is present at a concentration of at least 0.1 mM.

74. A complex for reinforcing a portion of an organism,
comprising cells and a component derived from the cells.

75. A complex according to item 74, which has biological
15 integration capability with surroundings.

76. A complex according to item 75, wherein the biological
integration capability include capability to adhere to
20 surrounding cells and/or extracellular matrices.

77. A complex according to item 74, which is substantially
made of cells and a material derived from the cells.

78. A complex according to item 74, which is substantially
made of cells and an extracellular matrix derived from the
25 cells.

79. A synthetic tissue according to item 78, wherein the
30 extracellular matrix is selected from the group consisting
of collagen I, collagen III, vitronectin and fibronectin.

80. A complex according to item 78, wherein the extracellular

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matrix and the cells are integrated together into a three-dimensional structure.

81. A complex according to item 78, wherein the extracellular matrix is provided on a surface of the complex.

82. A complex according to item 78, wherein the extracellular matrix is diffusely distributed on a surface of the complex.

83. A complex according to item 74, wherein an extracellular matrix is diffusely distributed on a surface of the complex, and the distribution densities of the extracellular matrix in two arbitrary sections of 1 cm² in the complex have a ratio within a range of about 1:3 to about 3:1.

84. A complex according to item 78, wherein the extracellular matrix includes fibronectin or vitronectin.

85. A complex according to item 74, which is heterologous, allogenic, isologous, or autogenous.

86. A complex according to item 74, wherein the portion includes a bag-shaped organ.

87. A complex according to item 86, wherein the bag-shaped organ includes a heart.

88. A complex according to item 74, wherein the portion includes a bone or cartilage tissue.

89. A complex according to item 74, wherein the portion includes avascular tissue.

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90. A complex according to item 74, wherein the portion includes an intervertebral disk, a meniscus, a ligament, or a tendon.
- 5 91. A complex according to item 74, wherein the reinforcement is achieved by replacing the portion with the complex or providing the complex to cover the portion, or both.
- 10 92. A complex according to item 74, which resists the expansion and contraction of the portion.
93. A complex according to item 74, which has biological integration.
- 15 94. A complex according to item 74, wherein the biological integration selected from the group consisting of internal binding of extracellular matrix, electrical integration, and intercellular signal transduction.
- 20 95. A complex according to item 74, which is formed by culturing cells in the presence of an ECM synthesis promoting agent.
- 25 96. A complex according to item 74, which has self-supporting ability.
97. A method for reinforcing a portion of an organism, comprising the steps of:
- 30 A) replacing the portion with a complex comprising cells and a component derived from the cells or providing the complex to cover the portion, or both; and
- B) holding the complex for a sufficient period of time for biologically adhering the complex to the portion.

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98. A method according to item 97, wherein the adhesion is achieved by adhesion between extracellular matrix and extracellular matrix.
99. A method according to item 97, which has biological integration capability with surroundings.
100. A method according to item 99, wherein the biological integration capability include capability to adhere to surrounding cells and/or extracellular matrices.
101. A method according to item 97, which is substantially made of cells and a material derived from the cells.
102. A method according to item 97, which is substantially made of cells and an extracellular matrix derived from the cells.
103. A method according to item 102, wherein the extracellular matrix contains one selected from the group consisting of collagen I, collagen III, vitronectin and fibronectin.
104. A method according to item 102, wherein the extracellular matrix contains all of collagen I, collagen III, vitronectin and fibronectin.
105. A method according to item 102, wherein the extracellular matrix contains vitronectin.
106. A method according to item 102, wherein the extracellular matrix contains fibronectin.

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107. A method according to item 97, wherein an extracellular matrix is provided on a surface of the complex.
- 5 108. A method according to item 97, wherein an extracellular matrix is diffusedly distributed on a surface of the complex.
109. A method according to item 97, wherein an extracellular matrix is diffusedly distributed on a surface of the complex,
10 and the distribution densities of the extracellular matrix in two arbitrary sections of 1 cm^2 have a ratio within a range of about 1:3 to about 3:1.
110. A complex according to item 97, wherein an extracellular
15 matrix is diffusedly distributed on a surface of the complex, and the distribution densities of the extracellular matrix in two arbitrary sections of 1 cm^2 have a ratio within a range of about 1:2 to about 2:1.
- 20 111. A method according to item 97, which is heterologous, allogenic, isologous, or autogenous.
112. A method according to item 97, wherein the portion
includes a bag-shaped organ.
- 25 113. A method according to item 112, wherein the bag-shaped organ includes a heart.
114. A method according to item 97, wherein the complex
30 resists the expansion and contraction of the portion.
115. A method according to item 97, wherein the complex has biological integration.

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116. A method according to item 115, wherein the biological
integration selected from the group consisting of internal
binding of extracellular matrix, electrical integration,
5 and intercellular signal transduction.

117. A method according to item 97, further comprising:
forming the complex by culturing the cells in the
presence of an ECM synthesis promoting agent.

10 118. A method according to item 97, wherein the portion is
a heart and the heart has a disease or disorder selected
from the group consisting of heart failure, ischemic heart
disease, myocardial infarct, cardiomyopathy, myocarditis,
15 hypertrophic cardiomyopathy, dilated phase hypertrophic
cardiomyopathy, and dilated cardiomyopathy.

119. A method according to item 97, wherein the portion
includes an avascular lesion.

20 120. A method according to item 97, wherein the portion
includes a vascular lesion.

121. A method according to item 97, wherein the portion
25 includes a bone or a cartilage.

122. A method according to item 97, wherein the portion
includes an intervertebral disk, a meniscus, a ligament,
or a tendon.

30 123. A method according to item 97, wherein the portion
includes a bone or a cartilage, and the bone or the cartilage
is damaged or degenerated.

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124. A method according to item 97, wherein the portion includes intractable fracture, osteonecrosis, cartilage injury, meniscus injury, ligament injury, tendon injury, 5 cartilage degeneration, meniscus degeneration, intervertebral disk denaturation, ligament degeneration, or tendon degeneration.
125. A method according to item 97, wherein the sufficient 10 period of time is at least 10 days.
126. A method according to item 97, wherein the complex has self-supporting ability.
- 15 127. A method according to item 97, which has Biological integration capability with surroundings.
128. A method according to item 97, which is substantially made of cells and an extracellular matrix derived from the 20 cells.
129. A method according to item 97, further comprising implanting another synthetic tissue.
- 25 130. A method according to item 129, wherein the other synthetic tissue is an artificial bone or a microfibrinous collagen medical device.
- 30 131. A method according to item 97, which is substantially made of cells and an extracellular matrix derived from the cells, wherein the other synthetic tissue is an artificial bone or a microfibrinous collagen medical device.

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132. A method according to item 130, the artificial bone includes hydroxyapatite.

5 133. A method for treating a portion of an organism, comprising the steps of:

A) replacing the portion with a complex comprising cells and a component derived from the cells or providing the complex to cover the portion, or both; and

10 B) holding the complex for a sufficient period of time for restoring a condition of the portion.

134. A method according to item 133, wherein the treatment is for the treatment, prevention, or reinforcement of a disease, disorder, or condition of a heart, a bone, a cartilage, a ligament, a tendon, a meniscus, of an
15 intervertebral disk.

135. A method according to item 133, wherein the complex has self-supporting ability.
20

136. A method according to item 133, wherein the complex has biological integration capability with surroundings.

25 137. A method according to item 133, wherein the complex is substantially made of cells and an extracellular matrix derived from the cells.

30 138. A method according to item 133, further comprising implanting another synthetic tissue in addition to the replacement or coverage of the portion.

139. A method according to item 138, wherein the other synthetic tissue includes an artificial bone or a

- 25 -

microfibrous collagen medical device.

140. A method according to item 133, which is substantially made of cells and an extracellular matrix derived from the cells, wherein the other synthetic tissue includes an artificial bone or a microfibrous collagen medical device.

141. A method according to item 139, the artificial bone includes hydroxyapatite.

142. A method for producing a synthetic tissue, comprising the steps of:

A) providing cells;

B) placing the cells in a container, the container having cell culture medium containing an ECM synthesis promoting agent and having a sufficient base area which can accommodate a synthetic tissue having a desired size;

C) culturing the cells in the container along with the cell culture medium containing the ECM synthesis promoting agent for a period of time sufficient for formation of the synthetic tissue having the desired size; and

D) regulating a thickness of the synthetic tissue by a physical or chemical stimulus to a desired thickness.

143. A method according to item 142, wherein the physical stimulus includes shear stress between the synthetic tissue and the container, deformation of the base of the container, shaking of the container, or pipetting.

144. A method according to item 142, wherein the chemical stimulus is obtained by using a chemical substance selected from the group consisting of actin depolymerizing agents and actin polymerizing agents.

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145. A method according to item 144, wherein the actin depolymerizing agent is selected from the group consisting of Slingshot, cofilin, CAP (cyclase associated protein), AIP1 (actininteractingprotein1), ADF (actindepolymerizing factor), destrin, depactin, actophorin, cytochalasin, and NGF (nerve growth factor).
146. A method according to item 144, wherein the actin polymerizing agent is selected from the group consisting of RhoA, mDi, profilin, Rac1, IRSp53, WAVE2, ROCK, LIM kinase, cofilin, cdc42, N-WASP, Arp2/3, Drf3, Mena, LPA (lysophosphatidic acid), insulin, PDGF (platelet derived growth factor), PDGFb, chemokine, and TGF (transforming growth factor) β .
147. A method according to item 144, wherein the desired thickness is regulated by adjusting a ratio of the actin depolymerizing agent to the actin polymerizing agent.
148. A method according to item 142, further comprising:
producing a plurality of the synthetic tissues and attaching the plurality of the synthetic tissues together to be integrated.
149. A tissue complex, comprising an implantable synthetic tissue and another synthetic tissue.
150. A tissue complex according to item 149, wherein the implantable synthetic tissue is substantially made of cells and a material derived from the cells.
151. A tissue complex according to item 149, wherein the

implantable synthetic tissue is substantially made of cells and an extracellular matrix derived from the cells.

5 152. A tissue complex according to item 151, wherein the extracellular matrix is selected from the group consisting of collagen I, collagen III, vitronectin, and fibronectin.

10 153. A tissue complex according to item 151, wherein the extracellular matrix contains all of collagen I, collagen III, vitronectin, and fibronectin.

15 154. A tissue complex according to item 149, wherein the other synthetic tissue includes an artificial bone or a microfibrinous collagen medical device.

155. A tissue complex according to item 154, the artificial bone includes hydroxyapatite.

20 156. A tissue complex according to item 149, the implantable synthetic tissue is biologically integrated with the other synthetic tissue.

25 157. A tissue complex according to item 156, wherein the biological integration is achieved via an extracellular matrix.

30 158. A composition for use in producing a synthetic tissue having a desired thickness, comprising a chemical substance selected from the group consisting of actin depolymerizing agents and actin polymerizing agents.

159. A composition according to item 158, wherein the actin depolymerizing agent is selected from the group consisting

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of Slingshot, cofilin, CAP (cyclase associated protein), AIP1 (actin interacting protein 1), ADF (actin depolymerizing factor), destrin, depactin, actophorin, cytochalasin, and NGF (nerve growth factor).

5

160. A composition according to item 158, wherein the actin polymerizing agent is selected from the group consisting of RhoA, mDi, profilin, Rac1, IRSp53, WAVE2, ROCK, LIM kinase, cofilin, cdc42, N-WASP, Arp2/3, Drf3, Mena, LPA

10 (lysophosphatidic acid), insulin, PDGF (platelet derived growth factor) α , PDGF β , chemokine, and TGF (transforming growth factor) β .

Hereinafter, the present invention will be described by way of preferable examples. It will be understood by those skilled in the art that the examples of the present invention can be appropriately made or carried out based on the description of the present specification and commonly used techniques well known in the art. The function and effect of the present invention can be easily recognized by those skilled in the art.

The present invention provides a scaffold-free synthetic tissue or complex. By providing such a scaffold-free synthetic tissue, a therapeutic method and a therapeutic agent for providing an excellent therapeutic result after implantation can be obtained.

The scaffold-free synthetic tissue of the present invention solves a long outstanding problem with biological formulations, which is attributed to contamination of the scaffold itself. Despite the absence of a scaffold, the therapeutic effect is comparable with, or more satisfactory

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- 29 -

than conventional techniques.

5 In addition, when a scaffold is used, the alignment of implanted cells in the scaffold, the cell-to-cell adhesion, the in vivo alteration of the scaffold itself (eliciting inflammation), the integration of the scaffold to recipient tissue, and the like become problematic. These problems can be solved by the present invention.

10 The synthetic tissue and the complex of the present invention are also self-organized, and have biological integration inside thereof. Also on this point, the present invention is distinguished from conventional cell therapies.

15 It is easy to form a three-dimensional structure with the synthetic tissue or complex of the present invention, and thus it is easy to design it into a desired form. The versatility of the synthetic tissue and the complex of the present invention should be noted.

20 The synthetic tissue and the complex of the present invention have biological integration with recipient tissues, such as adjacent tissues, cells, and the like. Therefore, the post-operational stability is satisfactory, and cells are securely supplied to a local site, for example. An effect of the present invention is that the satisfactory biological integration capability allows the formation of a tissue complex with another synthetic tissue or the like, resulting in a complicated therapy.

30 Another effect of the present invention is that differentiation can be induced after the synthetic tissue or the complex is provided. Alternatively, differentiation

- 30 -

is induced before providing a synthetic tissue and/or a complex, and thereafter, the synthetic tissue and/or the complex are developed.

5 Another effect of the present invention is that the implantation of the synthetic tissue of the present invention provides a satisfactory tissue replacement ability and a comprehensive supply of cells for filling or covering an implanted site, compared to conventional cell-only
10 implantation and sheet implantation.

 The present invention provides an implantable synthetic tissue with biological integration capability. The above-described features and effects of the present
15 invention make it possible to treat a site which cannot be considered as an implantation site for conventional synthetic products. The synthetic tissue of the present invention has biological integration and actually works in implantation
therapies. The synthetic tissue is for the first time provided
20 by the present invention, but is not provided by conventional techniques. The synthetic tissue or composite of the present invention has the sufficient ability to integrating with adjacent tissues, cells or the like during implantation (preferably, due to extracellular matrix). Therefore,
25 post-operational restoration is excellent. Such a synthetic tissue, which has biological integration capability in all of the three dimensions, cannot be achieved by conventional techniques. Therefore, the present
invention provides a therapeutic effect which cannot be
30 achieved by conventional synthetic tissue.

 In addition, the present invention provides medical treatment which provides a therapeutic effect by filling,

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replacing, and/or covering a lesion.

In addition, when the synthetic tissue of the present invention is used in combination with another synthetic tissue (e.g., an artificial bone made of hydroxyapatite, a microfibrous collagen medical device, etc.), the synthetic tissue of the present invention is biologically integrated with the other synthetic tissue, so that the acceptance of the synthetic tissue makes it possible to organize more complicated tissue complex which is not conventionally expected.

An extracellular matrix or a cell adhesion molecule, such as fibronectin, vitronectin, or the like, is distributed throughout the synthetic tissue of the present invention. In the cell sheet engineering, a cell adhesion molecule is localized on a bottom surface of culture cells which is attached to a Petri dish. In the sheet provided by the cell sheet engineering, cells are major components of the sheet. The sheet is intended to provide a mass of cells with an adhesion molecule attached on the bottom surface. The synthetic tissue of the present invention is a real "tissue" such that an extracellular matrix three-dimensionally integrates with cells. Thus, the present invention is significantly distinguished from conventional techniques including the cell sheet engineering.

A cell implanting method without a scaffold has been reported by a Tokyo Women's Medical University group, in which a cell sheet is produced using a temperature sensitive culture dish. Such a cell sheet engineering technique is internationally appraised due to its originality. However, a single sheet obtained by this technique is fragile. In

order to obtain the strength that can withstand surgical manipulation, such as implantation, a plurality of sheets need to be piled up, for example. Such a problem is solved by the present invention.

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A cell/matrix complex developed by the present invention does not require a temperature sensitive culture dish unlike the cell sheet technique. It is easy for the cell/matrix complex to form into a contractile
10 three-dimensional tissue. There is no technique in the world other than the present invention, which can produce a contractile three-dimensional complex having 10 or more layers of cells without using so-called feeder cells, such as rodent stroma cells, in about three weeks. By adjusting
15 conditions for matrix synthesis of the cell, it is possible to produce a complex having a strength which allows surgical manipulation, such as holding or transferring the complex, without a special instrument. Therefore, the present invention is an original, epoch-making technique in the world
20 for reliably and safely perform cell implantation.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows macroscopy and histology of exemplary
25 synthetic tissues using synovial cells.

Figure 2 shows high magnification histology of a synthetic tissue when ascorbic acid 2-phosphate has a concentration of 0 mM, 0.1 mM, 1 mM, and 5 mM. As can be
30 seen, Eosin staining of the synthetic tissue is more intense when ascorbic acid 2-phosphate is added at a concentration of more than 0.1 mM.

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Figure 3 shows a high magnification view of a synthetic tissue on day 3, 7, 14, and 21 of culture. As can be seen, the synthetic tissue is already developed at day 3 but the matrix is scarce. The matrix is getting dense with time.

Figure 4 shows an exemplary stained extracellular matrix in a synthetic tissue derived from synovial cells.

Figure 5 shows exemplary histology of normal tissue (normal skin tissue, synovial membrane tissue, tendon tissue, cartilage tissue, and meniscus tissue).

Figure 6 shows exemplary histology of a commercially available stained collagen sponge as a control. From the left, staining of fibronectin, vitronectin, non-IgG-immune as a negative control and HE staining are shown.

Figure 7 shows the results of collagen content measurement. When 0.1 mM or more of ascorbic acid diphosphate is added, collagen content in the synthetic tissue of the present invention is significantly increased in any of the culture periods. However, substantially no difference among the concentrations of 0.1 mM, 1 mM and 5 mM were found.

Figure 8 shows the results of collagen content measurement. When 0.1 mM or more of ascorbic acid diphosphate is added, collagen content in the synthetic tissue of the present invention is significantly increased in any of the culture periods. However, substantially no difference among the concentrations of 0.1 mM, 1 mM and 5 mM were found.

Figure 9 shows a production of synthetic tissues

using a different number of cells. F represents the number of passages. Numeral figures in the photograph indicate the number of cells per cm^2 .

5 Figure 10 shows a production of synthetic tissues using dishes with different sizes. * indicates culture in a 35-mm dish. ** indicates culture in a 60-mm dish. *** indicates culture in a 100-mm dish.

10 Figure 11 shows an exemplary mechanical testing system for measuring mechanical properties.

 Figure 12 shows a test piece holding portion of an apparatus for measuring mechanical properties.

15 Figure 13 shows an enlarged view of an apparatus for measuring mechanical properties. A test piece is provided with a marker.

20 Figure 14 shows an enlarged view of a test piece holding portion.

 Figure 15 shows a disrupted synthetic tissue after a tensile test.

25 Figure 16 shows the results (load-deformation curve) of a tensile test of a synthetic tissue (derived from synovium) of the present invention.

30 Figure 17 shows the results (stress-strain curve) of a mechanical properties test of a synthetic tissue (derived from synovial membrane tissue) of the present invention.

Figure 18 shows an exemplary osteogenic induction experiment of the synthetic tissue of the present invention and the results. The upper half portion shows a scheme for osteogenesis induction. The induction was conducted in the presence of 0.1 μ M dexamethasone, 10 mM β -glycerophosphate, and 50 μ g/ml ascorbic acid 2-phosphate. The lower left portion shows a control. The middle left portion shows a synthetic tissue differentiated into a bone by osteogenic induction. The middle lane portion shows Alizerin Red staining. The lower right portion shows an ALP-stained control. The middle right portion shows positive ALP-staining in a synthetic tissue by osteogenic induction.

Figure 19 shows the results of chondrogenic differentiation of a synthetic tissue of the present invention. This figure shows cultured synthetic tissues (A) and monolayer (B) using, from the leftmost, normal culture medium, chondrogenic medium, chondrogenic medium plus BFM-2 and chondrogenic medium plus TGF- β 1, respectively. Note that A) synthetic tissues have more intense staining of Alcian blue than B) monolayer culture. Also, note that addition of TGF- β results in detachment of a synthetic tissue from the container without mechanical stimulation. (A) Most right lane.

Figure 20 shows semi-quantification of Alcian blue staining for comparison of a synthetic tissue of the present invention with a single cell sheet under chondrogenic stimulation as in Figures 19 and 39. The left (blue) shows a result of monolayer, and the right (red) shows a result of the synthetic tissue.

Figure 21 shows the expression of various

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chondrogenic marker genes (aggrecan, Col II, Sox9, B-actin) under chondrogenic stimulation.

5 Figure 22 shows the comparison of the expression of chondrogenic marker genes within a synthetic tissue and a monolayer culture of synovial cells under chondrogenic stimulation as in Figures 19 and 21.

10 Figure 23 shows an *in vitro* cartilage implantation experiment using a synthetic tissue of the present invention and the results. The upper portion shows a diagram of explant culture. It is shown that a synthetic tissue is adhered to a partial thickness cartilage injury (*in vitro*). A superficial zone was removed, followed by digestion with chondroitinase ABC (Hinziker EB, JBJS, 1996). The lower left
15 portion is lower magnification histology ($\times 40$). The lower right portion is higher magnification histology ($\times 200$). As can be seen, the synthetic tissue is tightly attached to the injured surface.

20 Figure 24 shows an *in vivo* cartilage implantation experiment of the present invention and the 10 day results. A synthetic tissue is firmly adhered to a partial cartilage injury. The left shows a macroscopic view of the result. The upper right shows a histology ($\times 40$) and the lower right
25 shows a histology at higher magnification ($\times 200$).

Figure 25 shows the adhesion of a synthetic tissue of the present invention in a cartilage implantation experiment. The state on day 10 is shown. The left portion
30 shows the result of HE staining, the middle portion shows the result of fibronectin staining, and the right portion shows the result of vitronectin staining.

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Figure 26 shows the 1-month result of an in vivo implantation experiment of the present invention. A synthetic tissue is integrated with adjacent cartilage tissue without inflammation. Further, a superficial portion of the synthetic tissue contained a number of fibroblast-like cells (Figure 27), and a deep portion of the synthetic tissue contained a number of chondrocyte-like cells (Figure 28), indicating the chondrogenesis of the synthetic tissue after the implantation at particularly deep portions.

Figure 27 shows a superficial portion of a synthetic tissue at one month after implantation.

Figure 28 shows a deep portion of a synthetic tissue at one month after implantation.

Figure 29 shows the result of a meniscus repair experiment using a synthetic tissue of the present invention. The left portion of the figure shows that a medial femoral condyle bone and an anterior horn of medial meniscus are exposed. The right figure shows a 6.5-mm defect in a medial knee joint in the anterior horn of medial meniscus.

Figure 30 shows a meniscus repair procedure. The left portion shows a defect before the implantation of a synovial membrane-derived synthetic tissue (lower left). The right portion shows the defect after the implantation of the synovial membrane-derived synthetic tissue.

Figure 31 shows the results of a meniscus repair experiment using a synthetic tissue of the present invention. A visual inspection four weeks after operation is shown.

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The upper portion shows a state of a cartilage. It is shown that substantially no degeneration or injury due to friction or the like was found on the corresponding chondral surface, i.e., the meniscal defect was recovered. The lower left and right portions show a repaired defect.

Figure 32 shows the results of a meniscus repair experiment using a synthetic tissue of the present invention. The upper portion shows a macroscopic view. The lower left portion shows histology of a repaired tissue. The lower right portion shows histology of a border between the repaired tissue and its adjacent meniscus (magnification: x200).

Figure 33 shows an immunohistochemistry of a synthetic tissue derived from adipose tissue. From the left, H&E staining, fibronectin staining, and vitronectin staining.

Figure 34 shows the results of osteogenic or chondrogenic induction of a synthetic tissue derived from adipose tissue.

Figure 35 shows the results of a synthetic tissue with osteogenic induction when dexamethasone and β -glycerophosphate were added in culture medium prior to a detachment procedure.

Figure 36 shows the results of a synthetic tissue with osteogenic induction when dexamethasone and β -glycerophosphate were added in culture medium after a detachment procedure.

Figure 37 shows histology of biological integration of collagen gel containing synovial cells with cartilage after implantation. There is failure in integration observed (arrow).

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Figure 38 shows biological integration after implantation to a chondral defect when a synthetic tissue of the present invention was used. The biological integration is completely established.

10

Figure 39 shows the effect of TGF- β on the detachment of a synthetic tissue. Addition of TGF- β leads to active detachment of the synthetic tissue.

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Figure 40 shows a transition in contraction of a synthetic tissue of the present invention where dihydrochytchalasin or Y27632 was added or not. Data is shown in predetermined culture time intervals.

20

Figure 41 shows a photograph indicating adhesion of a synthetic tissue of the present invention with an artificial bone after fourteen days of culture in chondrogenic medium.

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Figure 42 shows histology of a synthetic tissue cultured on a collagen synthetic tissue (CMI collagen sponge, Amgen, USA), which is a microfibrillar collagen medical device, for 7 days.

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Figure 43 shows a skeletal muscle-derived sheet developed by a synthetic tissue production method without ascorbic acid.

Figure 44 shows a skeletal muscle-derived synthetic

- 40 -

tissue developed by a synthetic tissue production method with ascorbic acid according to the present invention.

Figure 45 shows histology of the synthetic tissue as shown in Figure 44 (HE staining).

Figure 46 shows a diagram for explaining a technique for measuring stress and distortion characteristics to determine tensile strength.

Figure 47 shows a principle for obtaining a load/removal of a load curve.

(Description of Sequencing List)

SEQ ID NO.: 1 indicates the nucleic acid sequence of myosin heavy chain IIa (human: Accession No. NM_017534).

SEQ ID NO.: 2 indicates the amino acid sequence of myosin heavy chain IIa (human: Accession No. NM_017534).

SEQ ID NO.: 3 indicates the nucleic acid sequence of myosin heavy chain IIb (human: Accession No. NM_017533).

SEQ ID NO.: 4 indicates the amino acid sequence of myosin heavy chain IIb (human: Accession No. NM_017533).

SEQ ID NO.: 5 indicates the nucleic acid sequence of myosin heavy chain IIc (IIx) (human: Accession No. NM_005963).

SEQ ID NO.: 6 indicates the amino acid sequence of myosin heavy chain IIc (IIx) (human: Accession

- 41 -

No. NM_005963).

SEQ ID NO.: 7 indicates the nucleic acid sequence of CD56 (human: Accession No. U63041).

5

SEQ ID NO.: 8 indicates the amino acid sequence of CD56 (human: Accession No. U63041).

10 SEQ ID NO.: 9 indicates the nucleic acid sequence of human MyoD (GENBANK Accession No. X56677).

SEQ ID NO.: 10 indicates a polypeptide sequence encoded by the nucleic acid sequence of SEQ ID NO.: 2.

15 SEQ ID NO.: 11 indicates the nucleic acid sequence of human myogenic factor 5 (MYF5) (GENBANK Accession No. NM_005593).

20 SEQ ID NO.: 12 indicates a polypeptide sequence encoded by the nucleic acid sequence of SEQ ID NO.: 3.

SEQ ID NO.: 13 indicates the nucleic acid sequence of human myogenin (myogenic factor 4) (GENBANK Accession No. BT007233).

25

SEQ ID NO.: 14 indicates a polypeptide sequence encoded by the nucleic acid sequence of SEQ ID NO.: 13.

30 SEQ ID NO.: 15 indicates the nucleic acid sequence of Sox9 (human: Accession No. NM_000346 = a marker specific to a chondrocyte).

SEQ ID NO.: 16 indicates a polypeptide sequence

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encoded by the nucleic acid sequence of SEQ ID NO.: 15.

SEQ ID NO.: 17 indicates the nucleic acid sequence
of Col 2A1 (human: Accession No. NM_001844 = a marker specific
5 to a chondrocyte).

SEQ ID NO.: 18 indicates a polypeptide sequence
encoded by the nucleic acid sequence of SEQ ID NO.: 17.

10 SEQ ID NO.: 19 indicates the nucleic acid sequence
of Aggrecan (human: Accession No. NM_001135 = a marker
specific to a chondrocyte).

SEQ ID NO.: 20 indicates a polypeptide sequence
15 encoded by the nucleic acid sequence of SEQ ID NO.: 19.

SEQ ID NO.: 21 indicates the nucleic acid sequence
of Bone sialoprotein (human: Accession No. NM_004967 = a
marker specific to an osteoblast).

20 SEQ ID NO.: 22 indicates a polypeptide sequence
encoded by the nucleic acid sequence of SEQ ID NO.: 21.

SEQ ID NO.: 23 indicates the nucleic acid sequence
25 of Osteocalcin (human: Accession No. NM_199173 = a marker
specific to an osteoblast).

SEQ ID NO.: 24 indicates a polypeptide sequence
encoded by the nucleic acid sequence of SEQ ID NO.: 23.

30 SEQ ID NO.: 25 indicates the nucleic acid sequence
of GDF5 (human: Accession No. NM_000557 = a marker specific
to a ligament cell).

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SEQ ID NO.: 26 indicates a polypeptide sequence encoded by the nucleic acid sequence of SEQ ID NO.: 25.

5 SEQ ID NO.: 27 indicates the nucleic acid sequence of Six1 (human: Accession No. NM_005982 = a marker specific to a ligament cell).

10 SEQ ID NO.: 28 indicates a polypeptide sequence encoded by the nucleic acid sequence of SEQ ID NO.: 27.

15 SEQ ID NO.: 29 indicates the nucleic acid sequence of Scleraxis (human: Accession No. BX000280 = a marker specific to a ligament cell).

 SEQ ID NO.: 30 indicates a polypeptide sequence encoded by the nucleic acid sequence of SEQ ID NO.: 29.

20 BEST MODE FOR CARRYING OUT THE INVENTION

 The present invention will be described below. It should be understood throughout the present specification that articles for singular forms include the concept of their plurality unless otherwise mentioned. Therefore, articles or adjectives for singular forms (e.g., "a", "an", "the", and the like in English) include the concept of their plurality unless otherwise specified. Also, it should be also understood that terms as used herein have definitions ordinarily used in the art unless otherwise mentioned. Therefore, all technical and scientific terms used herein have the same meanings as commonly understood by those skilled in the relevant art. Otherwise, the present application (including definitions) takes precedence.

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(Definition of terms)

The definitions of specific terms used herein are described below.

5

(Regenerative medicine)

As used herein, the term "regeneration" refers to a phenomenon in which when an individual organism loses a portion of tissue, the remaining tissue grows and recovers. The extent or manner of regeneration varies depending among animal species or among tissues in the same individual. Most human tissues have limited regeneration capability, and therefore, complete regeneration is not expected if a large portion of tissue is lost. In the case of severe damage, a tissue may grow which has strong proliferation capability different from that of lost tissue, resulting in incomplete regeneration where the damaged tissue is incompletely regenerated and the function of the tissue cannot be recovered. In this case, a structure made of a bioabsorbable material is used to prevent a tissue having strong proliferation capability from infiltrating the injured portion of the tissue so as to secure space for proliferation of the damaged tissue. Further, by supplementing with a cell growth factor, the regeneration capability of the damaged tissue is enhanced. Such a regeneration technique is applied to cartilages, bones, hearts, and peripheral nerves, for example. It has been so far believed that cartilages, nerve cells, and cardiac muscles have no or poor regeneration capability. Recently, it was reported that there are tissue (somatic stem cells), which have both the capability of differentiating into these tissues and self-proliferation capability. Expectations are running high for regenerative medicine using stem cells. Embryonic stem cells (ES cells) also have the capability

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- 45 -

of differentiating into all tissues. Efforts have been made to use ES cells for regeneration of complicated organs, such as kidney, liver, and the like, but have not yet been realized.

5 The term "cell" is herein used in its broadest sense in the art, referring to a structural unit of tissue of a multicellular organism, which is capable of self-replicating, has genetic information and a mechanism for expressing it, and is surrounded by a membrane structure which isolates
10 the living body from the outside. In the method of the present invention, any cell can be used as a subject. The number of cells used in the present invention can be counted through an optical microscope. When counting using an optical microscope, the number of nuclei is counted. Tissues are
15 sliced into tissue sections, which are then stained with hematoxylin-eosin (HE) to variegate nuclei derived from extracellular matrices (e.g., elastin or collagen) and cells. These tissue sections are observed under an optical microscope and the number of nuclei in a particular area
20 (e.g., $200\ \mu\text{m} \times 200\ \mu\text{m}$) can be estimated to be the number of cells. Cells used herein may be either naturally-occurring cells or artificially modified cells (e.g., fusion cells, genetically modified cells, etc.). Examples of cell sources include, but are not limited to,
25 a single-cell culture; the embryo, blood of a normally-grown transgenic animal; a cell mixture of cells derived from normally-grown cell lines; and the like. Primary culture cells may be used. Alternatively, subculture cells may also be used. Preferably, when subculture cells are used, the
30 cells are preferably of 3 to 8 passages. As used herein, cell density may be represented by the number of cells per unit area (e.g., cm^2).

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As used herein, the term "stem cell" refers to a cell capable of self replication and pluripotency. Typically, stem cells can regenerate an injured tissue. Stem cells used herein may be, but are not limited to, embryonic stem (ES) cells or tissue stem cells (also called tissular stem cell, tissue-specific stem cell, or somatic stem cell). A stem cell may be an artificially produced cell (e.g., fusion cells, reprogrammed cells, or the like used herein) as long as it can have the above-described abilities. Embryonic stem cells are pluripotent stem cells derived from early embryos. An embryonic stem cell was first established in 1981, and has been applied to production of knockout mice since 1989. In 1998, a human embryonic stem cell was established, which is currently becoming available for regenerative medicine. Tissue stem cells have a relatively limited level of differentiation unlike embryonic stem cells. Tissue stem cells are present in tissues and have an undifferentiated intracellular structure. Tissue stem cells have a higher nucleus/cytoplasm ratio and have few intracellular organelles. Most tissue stem cells have pluripotency, a long cell cycle, and proliferative ability beyond the life of the individual. As used herein, stem cells may be preferably embryonic stem cells, though tissue stem cells may also be employed depending on the circumstance.

Tissue stem cells are separated into categories of sites from which the cells are derived, such as the dermal system, the digestive system, the bone marrow system, the nervous system, and the like. Tissue stem cells in the dermal system include epidermal stem cells, hair follicle stem cells, and the like. Tissue stem cells in the digestive system include pancreatic (common) stem cells, hepatic stem cells, and the like. Tissue stem cells in the bone marrow system

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include hematopoietic stem cells, mesenchymal stem cells, and the like. Tissue stem cells in the nervous system include neural stem cells, retinal stem cells, and the like.

5 As used herein, the term "somatic cell" refers to any cell other than a germ cell, such as an egg, a sperm, or the like, which does not transfer its DNA to the next generation. Typically, somatic cells have limited or no pluripotency. Somatic cells used herein may be
10 naturally-occurring or genetically modified as long as they can achieve the intended treatment.

 The origin of a stem cell is categorized into the ectoderm, endoderm, or mesoderm. Stem cells of ectodermal
15 origin are mostly present in the brain, including neural stem cells. Stem cells of endodermal origin are mostly present in bone marrow, including blood vessel stem cells, hematopoietic stem cells, mesenchymal stem cells, and the like. Stem cells of mesoderm origin are mostly present in
20 organs, including hepatic stem cells, pancreatic stem cells, and the like. As used herein, somatic cells may be derived from any mesenchyme. Preferably, somatic cells derived from mesenchyme may be employed.

25 As cells for use in construction of a synthetic tissue or three-dimensional structure of the present invention, differentiated cells or stem cells derived from the above-described ectoderm, endoderm, or mesoderm may be employed, for example. Examples of such cells include
30 mesenchymal cells. In a certain embodiment, as such cells, myoblasts (e.g., skeletal myoblast, etc.), fibroblasts, synovial cells, and the like may be employed. As such cells, differentiated cells or stem cells can be used as they are.

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Cells differentiated from stem cells into a desired direction can be used.

As used herein, the term "mesenchymal stem cell" refers to a stem cell found in mesenchyme. The term "mesenchymal stem cell" may be herein abbreviated as "MSC". Mesenchyme refers to a population of free cells which are in the asterodal shape or have irregular projections and bridge gaps between epithelial tissues, and which are recognized in each stage of development of multicellular animals. Mesenchyme also refers to tissue formed with intracellular cement associated with the cells. Mesenchymal stem cells have proliferation ability and the ability to differentiate into osteocytes, chondrocytes, muscle cells, stroma cells, tendon cells, and adipocytes. Mesenchymal stem cells are employed in order to culture or grow bone marrow cells or the like collected from patients, or differentiate them into chondrocytes or osteoblasts. Mesenchymal stem cells are also employed as reconstruction material, such as alveolar bones, bones, cartilages or joints for arthropathy or the like; and the like. There is a large demand for mesenchymal stem cells. A synthetic tissue or three-dimensional structure of the present invention comprising mesenchymal stem cells or differentiated mesenchymal stem cells is particularly useful when a structure is required in these applications.

As used herein, the term "isolated" means that naturally accompanying material is at least reduced, or preferably substantially completely eliminated, in normal circumstances. Therefore, the term "isolated cell" refers to a cell substantially free of other accompanying substances (e.g., other cells, proteins, nucleic acids, etc.) in natural

circumstances. The term "isolated tissue" refers to a tissue substantially free of substances other than that tissue (e.g., in the case of synthetic tissues or complexes, substances, scaffolds, sheets, coats, etc. used when the synthetic tissue is produced). As used herein, the term "isolated" refers to a scaffold-free state. Therefore, it will be understood that the synthetic tissue or complex of the present invention in the isolated state may contain components (e.g., medium, etc.) used in the production of it. The term "isolated" in relation to nucleic acids or polypeptides means that, for example, the nucleic acids or the polypeptides are substantially free of cellular substances or culture media when they are produced by recombinant DNA techniques; or precursory chemical substances or other chemical substances when they are chemically synthesized. Isolated nucleic acids are preferably free of sequences naturally flanking the nucleic acid within an organism from which the nucleic acid is derived (i.e., sequences positioned at the 5' terminus and the 3' terminus of the nucleic acid).

As used herein, the term "scaffold-free" indicates that a synthetic tissue does not substantially contain a material (scaffold) which is conventionally used for production of a synthetic tissue. Examples of such a scaffold include, but are not limited to, chemical polymeric compounds, ceramics, or biological formulations such as polysaccharides, collagens, gelatins, hyaluronic acids, and the like. A scaffold is a material which is substantially solid and has a strength which allows it to support cells or tissue.

As used herein, the term "established" in relation to cells refers to a state of a cell in which a particular

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property (pluripotency) of the cell is maintained and the cell undergoes stable proliferation under culture conditions. Therefore, established stem cells maintain pluripotency.

5 As used herein, the term "non-embryonic" refers to not being directly derived from early embryos. Therefore, the term "non-embryonic" refers to cells derived from parts of the body other than early embryos. Also, modified embryonic stem cells (e.g., genetically modified or fusion
10 embryonic stem cells, etc.) are encompassed by non-embryonic cells.

 As used herein, the term "differentiated cell" refers to a cell having a specialized function and form (e.g., muscle
15 cells, neurons, etc.). Unlike stem cells, differentiated cells have no or little pluripotency. Examples of differentiated cells include epidermic cells, pancreatic parenchymal cells, pancreatic duct cells, hepatic cells, blood cells, cardiac muscle cells, skeletal muscle cells,
20 osteoblasts, skeletal myoblasts, neurons, vascular endothelial cells, pigment cells, smooth muscle cells, adipocytes, osteocytes, chondrocytes, and the like.

 As used herein, the term "tissue" refers to a group
25 of cells having the same function and form in cellular organisms. In multicellular organisms, constituent cells are usually differentiated so that the cells have specialized functions, resulting in division of labor. Therefore, multicellular organisms are not simple cell aggregations,
30 but constitute organic or social cell groups having a certain function and structure. Examples of tissues include, but are not limited to, integument tissue, connective tissue, muscular tissue, nervous tissue, and the like. Tissue

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targeted by the present invention may be derived from any organ or part of an organism. In a preferable embodiment of the present invention, tissue targeted by the present invention includes, but is not limited to, a bone, a cartilage, a tendon, a ligament, a meniscus, an intervertebral disk, a pericosteum, a blood vessel, a blood vessel-like tissue, a heart, a cardiac valve, a pericardium, a dura mater, and the like.

As used herein, the term "cell sheet" refers to a structure comprising a monolayer of cells. Such a cell sheet has at least a two-dimensional biological integration. The sheet having biological integration is characterized in that after the sheet is produced, the connection between cells is not substantially destroyed even when the sheet is handled singly. Such biological integration includes intracellular connection via an extracellular matrix. It will be understood that the cell sheet may partially include a two or three-layer structure.

As used herein, the term "synthetic tissue" refers to tissue having a state different from natural states. Typically, a synthetic tissue is herein prepared by cell culture. Tissue which is removed from an organism and is not subjected to any treatment is not referred to as a synthetic tissue. Therefore, a synthetic tissue may include materials derived from organisms and materials not derived from organisms. The synthetic tissue of the present invention typically comprises a cell and/or a biological material, and may comprise other materials. More preferably, a synthetic tissue of the present invention is composed substantially only of a cell and/or a biological material. Such a biological material is preferably derived from cells

constituting the tissue (e.g., extracellular matrix, etc.).

As used herein, the term "implantable synthetic tissue" refers to a synthetic tissue, which can be used for actual clinical implantation and can function as a tissue at the implantation site for a certain period of time after implantation. Implantable synthetic tissue typically has sufficient biocompatibility, sufficient affinity, and the like.

The sufficient strength of an implantable synthetic tissue varies depending on a part targeted by implantation, but can be determined as appropriate by those skilled in the art. The strength is sufficient to provide self-supporting ability, and can be determined depending on the environment of implantation. The strength can be measured by measuring stress or distortion characteristics or conducting a creep characteristics indentation test as described below. The strength may also be evaluated by observing the maximum load.

The sufficient size of an implantable synthetic tissue varies depending on a part targeted by implantation, but can be determined as appropriate by those skilled in the art. The size can be determined depending on the environment of implantation.

However, an implantable synthetic tissue preferably has at least a certain size. Such a size (e.g., area) is at least 1 cm², preferably at least 2 cm², more preferably at least 3 cm², even more preferably at least 4 cm², at least 5 cm², at least 6 cm², at least 7 cm², at least 8 cm², at least 9 cm², at least 10 cm², at least 15 cm², or at least 20 cm².

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An essence of the present invention is that a synthetic tissue of any size (area, volume) can be produced, i.e., the size is not particularly limited.

5 When the size is represented by the volume, the size may be, but is not limited to, at least 2 mm³, or at least 40 mm³. The size may be 2 mm³ or less or 40 mm³ or more.

10 The sufficient thickness of an implantable synthetic tissue varies depending on a part targeted by implantation, but can be determined as appropriate by those skilled in the art. The thickness can be determined depending on the environment of implantation. The thickness may exceed 5 mm. When an implantable synthetic tissue is implanted into the
15 heart, the tissue may only have these minimum thicknesses. When implantable synthetic tissue is used in other applications, the tissue may preferably have a greater thickness. In such a case, for example, an implantable synthetic tissue has preferably a thickness of at least 2 mm,
20 more preferably at least 3 mm, and even more preferably 5 mm. For example, when an implantable synthetic tissue is applied to a bone, a cartilage, a ligament, a tendon, or the like, similar to the case of a heart, the tissue has a thickness of at least about 1 mm (e.g., at least 2 mm, more preferably
25 at least 3 mm, and even more preferably 5 mm), or 5 mm or more or less than 1 mm. An essence of the present invention is that a synthetic tissue or complex of any thickness can be produced, i.e., the size is not particularly limited.

30 The sufficient biocompatibility of implantable synthetic tissue varies depending on a part targeted by implantation, but can be determined as appropriate by those skilled in the art. However, an implantable synthetic tissue

preferably has at least a certain level of biocompatibility. Typically, a desired level of biocompatibility is, for example, such that biological integration to surrounding tissues is achieved without any inflammation, any immune reaction or the like. The present invention is not limited to this. In some cases (e.g., corneas, etc.), an immune reaction is less likely to occur. Therefore, an implantable synthetic tissue has biocompatibility to an extent, which achieves the object of the present invention even when an immune reaction is likely to occur in other organs. Examples of parameters indicating biocompatibility include, but are not limited to, the presence or absence of an extracellular matrix, the presence or absence of an immune reaction, the degree of inflammation, and the like. Such biocompatibility can be determined by examining the compatibility of a synthetic tissue at an implantation site after implantation (e.g., confirming that an implanted synthetic tissue is not destroyed). See "Hito Ishoku Zoki Kyozeitsu Hanno no Byori Soshiki Shindan Kijyun Kanbetsu Shindan to Seiken Hyohon no Toriatukai (Zufu) Jinzo Ishoku, Kanzo Ishoku Oyobi Shinzo Ishoku [Pathological Tissue Diagnosis Criterion for Human Transplanted Organ Rejection Reaction Handling of Differential Diagnosis and Biopsy Specimen (Illustrated Book) Kidney Transplantation, Liver Transplantation and Heart Transplantation]" The Japan Society for Transplantation and The Japanese Society for Pathology editors, Kanehara Shuppan Kabushiki Kaisha (1998). According to this document, biocompatibility is divided into Grade 0, 1A, 1B, 2, 3A, 3B, and 4. At Grade 0 (no acute rejection), no acute rejection reaction, cardiomyocyte failure, or the like is found in biopsy specimens. At Grade 1A (focal, mild acute rejection), there is focal infiltration of large lymphocytes around blood vessels or into

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interstitial tissue, while there is no damage to cardiomyocytes. This observation is obtained in one or a plurality of biopsy specimens. At Grade 1B (diffuse, mild acute rejection), there is diffuse infiltration of large lymphocytes around blood vessels or into interstitial tissue or both, while there is no damage to cardiomyocytes. At Grade 2 (focal, moderate acute rejection), there is a single observed infiltration focus of inflammatory cells clearly bordered from the surrounding portions. Inflammation cells are large activated lymphocytes and may include eosinophils. Damage to cardiomyocytes associated with modification of cardiac muscle is observed in lesions. At Grade 3A (multifocal, moderate acute rejection), there are multiple infiltration foci of inflammatory cells which are large activated lymphocytes and may include eosinophils. Two or more of the multiple inflammatory infiltration foci of inflammatory cells have damages to cardiomyocytes. In some cases, there is also rough infiltration of inflammatory cells into the endocardium. The infiltration foci are observed in one or a plurality of biopsy specimens. At Grade 3B (multifocal, borderline severe acute rejection), there are more confluent and diffuse infiltration foci of inflammatory cells found in more biopsy specimens than those observed at Grade 3A. There is infiltration of inflammatory cells including large lymphocytes and eosinophils, in some cases neutrophils, as well as damage to cardiomyocytes. There is no hemorrhage. At Grade 4 (severe acute rejection), there is infiltration of various inflammatory cells including activated lymphocytes, eosinophils, and neutrophils. There is always damage to cardiomyocytes and necrosis of cardiomyocytes. Edema, hemorrhage, and/or angitis are also typically observed. Infiltration of inflammatory cells into the endocardium, which is different from the "Quilty"

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effect, is typically observed. When a therapy is strongly conducted using an immunosuppressant for a considerably long period of time, edema and hemorrhage may be more significant than infiltration.

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The sufficient affinity of an implantable synthetic tissue varies depending on a part targeted by implantation, but can be determined as appropriate by those skilled in the art. Examples of parameters for affinity include, but
10 are not limited to, biological integration capability between an implanted synthetic tissue and its implantation site, and the like. Such affinity can be determined based on the presence of biological integration at an implantation site after implantation. Preferable affinity is herein such that
15 an implanted synthetic tissue has the same function as that of a site in which the tissue is implanted, for example.

As used herein, the term "self-supporting ability" in relation to a tissue (e.g., a synthetic tissue, etc.)
20 refers to a property of the synthetic tissue such that when it is restrained on at least one point thereof, it is not substantially destroyed. Self-supporting ability is herein observed if a tissue (e.g., a synthetic tissue) is picked up by using forceps with a tip having a thickness of 0.5
25 to 3.0 mm (preferably, forceps with a tip having a thickness of 1 to 2 mm or 1 mm; the forceps preferably have a bent tip) and the tissue is not substantially destroyed. Such forceps are commercially available (e.g., from Natsume Seisakusho, etc.). A force exerted for picking up a tissue
30 is comparable with a force typically exerted by a medical practitioner handling a tissue. Therefore, the self-supporting ability of a tissue can also be represented by a property such that the tissue is not destroyed when

it is picked up by a hand. Such forceps are, for example, but are not limited to, a pair of curved fine forceps (e.g., No. A-11 (tip: 1.0 mm in thickness) and No. A-12-2 (tip: 0.5 mm in thickness) commercially available from Natsume Seisakusho). A bent tip is suitable for picking up a synthetic tissue. The forceps are not limited to a bent tip type.

When a joint is treated, replacement is majorly performed. The strength of a synthetic tissue of the present invention required in such a case is such that a minimum self-supporting ability is obtained. Cells contained in the synthetic tissue are subsequently replaced with cells in an affected portion. The replacing cells produce a matrix which enhances the mechanical strength, so that the joint is healed. It will also be understood that the present invention may be used in conjunction with an artificial joint.

In the present invention, self-supporting ability plays an important role in evaluating the supporting ability of a synthetic tissue which is actually produced. When a synthetic tissue of the present invention is produced, the synthetic tissue is formed in the shape of a cell sheet in a container. Thereafter, the sheet is detached. With conventional techniques, the sheet is usually destroyed due to lack of self-supporting ability. Therefore, in conventional technology, an implantable synthetic tissue cannot be substantially produced. Especially, when a large-sized synthetic tissue is required, conventional techniques are not adequate. According to the technique of the present invention, a synthetic tissue can be produced, which has a sufficient strength which allows the tissue to be detached from a container without being destroying, i.e.,

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the tissue already has self-supporting ability when being detached. This is true even when the synthetic tissue is in the form of a monolayer sheet before being detached. It will be understood that the monolayer may partially include a two or three-layer structure. Thus, it will be understood that the synthetic tissue of the present invention is applicable in substantially any chosen therapy. In addition, typically, after a synthetic tissue is produced and detached, the strength and self-supporting ability of the synthetic tissue are increased as observed in the present invention. Therefore, in the present invention, it will be understood that the self-supporting ability evaluated upon production may be an important aspect. In the present invention, the strength upon implantation is also important. It may also be important to evaluate the self-supporting ability of a synthetic tissue when a predetermined time has passed after the production of the tissue. Therefore, it will be understood that the self supporting ability at the time of implantation after transport, can be determined by calculating the time that has elapsed since production of the tissue, based on the above-described relationship.

As used herein, the term "membranous tissue" refers to a tissue in the form of membrane and is also referred to as "planar tissue". Examples of membranous tissue include tissues of organs (e.g., periosteum, pericardium, duramater, cornea, etc.).

As used herein, the term "organ" refers to a structure which is a specific part of an individual organism where a certain function of the individual organism is locally performed and which is morphologically independent. Generally, in multicellular organisms (e.g., animals and

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plants), organs are made of several tissues in specific spatial arrangement and tissue is made of a number of cells. Examples of such organs include, but are not limited to, skin, blood vessel, cornea, kidney, heart, liver, umbilical cord, intestine, nerve, lung, placenta, pancreas, brain, joint, bone, cartilage, peripheral limbs, retina, and the like. Examples of such organs include, but are not limited to, organs of the skin system, the parenchyma pancreas system, the pancreatic duct system, the hepatic system, the blood system, the myocardial system, the skeletal muscle system, the osteoblast system, the skeletal myoblast system, the nervous system, the blood vessel endothelial system, the pigment system, the smooth muscle system, the fat system, the bone system, the cartilage system, and the like.

As used herein, the term "bag-shaped organ" refers to an organ which has a three-dimensional expanse and the inside of which may be connected via a tubular tissue to the outside. Examples of bag-shaped organs include, but are not limited to, heart, liver, kidney, stomach, spleen, and the like.

In one embodiment, the present invention targets an intervertebral disk, a cartilage, a joint, a bone, a meniscus, a synovial membrane, a ligament, a tendon, and the like. In a preferable embodiment, the present invention targets blood vessels, blood vessel-like tissue, heart, heart valves, pericardia, dura mater, cornea, and bones. In another preferable embodiment, examples of organs targeted by the present invention include, but are not limited to, skeletal muscle, fat, and the like in addition to what is described above.

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As used herein, the term "cover" or "wrap" in relation to a synthetic tissue, a three-dimensional structure, or the like, which is wrapped around a certain part (e.g., an injured site, etc.), means that the synthetic tissue or the like is arranged so as to cover the part (i.e., conceal an injury or the like). The terms "wrap" and "arrange (or locate) so as to cover" are used interchangeably. By observing the spatial relationship between the part and the synthetic tissue or the like, it can be determined whether or not the part is covered by the synthetic tissue or the like. In a preferable embodiment, in a covering step, a synthetic tissue or the like can be wrapped one turn around a certain site.

As used herein, the term "replace" means that a lesion (a site of an organism) is replaced, and cells which have originally been in a lesion are replaced with cells supplied by a synthetic tissue or a complex according to the present invention. Examples of a disease for which replacement is suitable include, but not limited to, a ruptured site, and the like. The term "fill" may be used in place of the term "replace" in the present specification.

A "sufficient time required for a synthetic tissue to biologically integrate with a part" herein varies depending on a combination of the part and the synthetic tissue, but can be determined as appropriate by those skilled in the art based on the combination. Examples of such a time include, but are not limited to, 1 week, 2 weeks, 1 month, 2 months, 3 months, 6 months, 1 year, and the like, after operation. In the present invention, a synthetic tissue preferably comprises substantially only cells and materials derived from the cells, and therefore, there is no particular

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material which needs to be extracted after operation. Therefore, the lower limit of the sufficient time is not particularly important. Thus, in this case, a longer time is more preferable. If the time is substantially extremely
5 long, reinforcement is substantially completed.

As used herein, the term "immune reaction" refers to a reaction due to the dysfunction of immunological tolerance between a graft and a host. Examples of immune
10 reactions include, but are not limited to, a hyperacute rejection reaction (within several minutes after implantation) (immune reaction caused by antibodies, such as β -Gal or the like), an acute rejection reaction (reaction caused by cellular immunity about 7 to 21 days after
15 implantation), a chronic rejection reaction (rejection reaction caused by cellular immunity 3 or more months after operation), and the like.

As used herein, the elicitation of an immune reaction
20 can be confirmed by pathological and histological examination of the type, number, or the like of infiltration of (immunological) cells into implanted tissue using staining (e.g., HE staining, etc.), immunological staining, or microscopic inspection of tissue sections.

25 As used herein, the term "calcification" refers to precipitation of calcareous substances in organisms.

"Calcification" *in vivo* can be determined herein by
30 staining (e.g., Alizarin Red staining) and measuring calcium concentration. Specifically, implanted tissue is taken out; the tissue section is dissolved by acid treatment or the like; and the atomic absorption of the solution is measured

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by a trace element quantifying device.

As used herein, the term "within organism(s)" (or in
organism(s))" or "in vivo" refers to the inner part of
5 organism(s). In a specific context, "within organism(s)"
refers to a position at which a subject tissue or organ is
placed.

As used herein, "in vitro" indicates that a part of
10 an organism is extracted or released outside the organism
for various purposes of research (e.g., in a test tube).
The term *in vitro* is in contrast to the term *in vivo*.

As used herein, the term "ex vivo" refers to a series
15 of operations where target cells into which a gene will be
introduced are extracted from a subject; a therapeutic gene
is introduced *in vitro* into the cells; and the cells are
returned into the same subject.

As used herein, the term "material derived from
20 cell(s)" refers to any material originating from the cell(s),
including, but not being limited to, materials constituting
the cell(s), materials secreted by the cell(s), materials
metabolized by the cell(s), and the like. Representative
25 examples of materials derived from cells include, but are
not limited to, extracellular matrices, hormones, cytokines,
and the like. Materials derived from cells typically have
substantially no adverse effect on the cells and their hosts.
Therefore, when the material is contained in a synthetic
30 tissue, a three-dimensional structure, or the like, the
material typically has substantially no adverse effect on
the synthetic tissue, three-dimensional structure, or the
like.

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As used herein, the term "extracellular matrix" (ECM) refers to a substance existing between somatic cells no matter whether the cells are epithelial cells or non-epithelial cells. Extracellular matrices are typically produced by cells, and therefore, are biological materials. Extracellular matrices are involved in supporting tissue as well as in internal environmental structure essential for survival of all somatic cells. Extracellular matrices are generally produced from connective tissue cells. Some extracellular matrices are secreted from cells possessing basal membrane, such as epithelial cells or endothelial cells. Extracellular matrices are roughly divided into fibrous components and matrices filling there between. Fibrous components include collagen fibers and elastic fibers. A basic component of matrices is a glycosaminoglycan (acidic mucopolysaccharide), most of which is bound to non-collagenous protein to form a polymer of a proteoglycan (acidic mucopolysaccharide-protein complex). In addition, matrices include glycoproteins, such as laminin of basal membrane, microfibrils around elastic fibers, fibers, fibronectins on cell surfaces, and the like. Particularly differentiated tissue has the same basic structure. For example, in hyaline cartilage, chondroblasts characteristically produce a large amount of cartilage matrices including proteoglycans. In bones, osteoblasts produce bone matrices which cause calcification. Herein, examples of typical extracellular matrix include, but not limited to, collagen I, collagen III, collagen V, elastin, vitronectin, fibronectin, proteoglycans (for example, decorin, biglycan, fibromodulin, lumican, hyaluronic acid, etc.). Various types of extracellular matrix may be utilized in the present invention as long as cell adhesion is achieved.

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In one embodiment of the present invention, the synthetic tissue, three-dimensional structure, or the like of the present invention may be advantageously similar to the composition of an extracellular matrix (e.g., elastin, collagen (e.g., Type I, Type III, Type IV, etc.), laminin, etc.) of a site of an organ for which implantation is intended. In the present invention, extracellular matrices include cell adhesion molecules. As used herein, the terms "cell adhesion molecule" and "adhesion molecule" are used interchangeably, referring to a molecule capable of mediating the joining of two or more cells (cell adhesion) or adhesion between a substrate and a cell. In general, cell adhesion molecules are divided into two groups: molecules involved in cell-cell adhesion (intercellular adhesion) (cell-cell adhesion molecules) and molecules involved in cell-extracellular matrix adhesion (cell-substrate adhesion) (cell-substrate adhesion molecules). A synthetic tissue or three-dimensional structure of the present invention typically comprises such a cell adhesion molecule. Therefore, cell adhesion molecules herein include a protein of a substrate and a protein of a cell (e.g., integrin, etc.) in cell-substrate adhesion. A molecule other than proteins falls within the concept of cell adhesion molecule as long as it can mediate cell adhesion.

It should be noted that the synthetic tissue or complex of the present invention comprises cells and a material (natively) derived from the cell. Therefore, such materials including ECMs form a complicated composition containing collagen I, collagen III, collagen V, elastin, fibronectin, vitronectin, proteoglycans (for example, decorin, biglycan, fibromodulin, lumican, hyaluronic acid,

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etc.). Conventionally a synthetic tissue containing such cell-derived ingredients has not been provided. To obtain a synthetic tissue having such a composition is substantially impossible when an artificial material is used. Thus, a composition containing such ingredients (particularly , collagen I, collagen III and the like) is recognized to be a native composition.

More preferably, an extracellular matrix includes all the collagen (for example, Types I, Type III, etc.), vitronectin, and fibronectin. Especially, a synthetic tissue containing vitronectin and/or fibronectin has not been provided before. Therefore, the synthetic tissue and the complex according to the present invention are recognized to be new in this regard.

As used herein, the term "provided" or "distributed" in relation to an extracellular matrix and the synthetic tissue of the present invention indicates that the extracellular matrix is present in the synthetic tissue. It should be understood that such superficial provision can be visualized and observed by immunologically staining an extracellular matrix of interest.

As used herein, the term "in a diffused manner" or "diffusedly" in relation to the distribution of an extracellular matrix indicates that the extracellular matrix is not localized. Such distribution of an extracellular matrix has a ratio of the distribution densities of two arbitrary sections of 1 cm^2 within a range of typically about 1:10 to about 10:1, and representatively about 1:3 to about 3:1, and preferably about 1:2 to about 2:1, and more preferably about 1:1 (i.e., substantially evenly distributed over the

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synthetic tissue. When an extracellular matrix is distributed on a surface of the synthetic tissue of the present invention, but not localized, the synthetic tissue of the present invention has biological integration capability evenly with respect to the surrounding. Therefore, the synthetic tissue of the present invention has an excellent effect of recovery after implantation.

For cell-cell adhesion, cadherin, a number of molecules belonging in an immunoglobulin superfamily (NCAM1, ICAM, fasciolin II, III, etc.), selectin, and the like are known, each of which is known to join cell membranes via a specific molecular reaction. Therefore, in one embodiment, the synthetic tissue, three-dimensional structure, or the like of the present invention preferably has substantially the same composition of cadherin, immunoglobulin superfamily molecules, or the like as that of a site for which implantation is intended.

Thus, various molecules are involved in cell adhesion and have different functions. Those skilled in the art can appropriately select a molecule to be contained in a synthetic tissue or three-dimensional structure of the present invention depending on the purpose. Techniques for cell adhesion are well known as described above and as described in, for example, "Saibogaimatorikkusu -Rinsho heno Oyo- [Extracellular matrix -Clinical Applications-], Medical Review.

It can be determined whether or not a certain molecule is a cell adhesion molecule, by an assay, such as biochemical quantification (an SDS-PAGE method, a labeled-collagen method, etc.), immunological quantification (an enzyme antibody

method, a fluorescent antibody method, an immunohistological study, etc.), a PCR method, a hybridization method, or the like, in which a positive reaction is detected. Examples of such a cell adhesion molecule include, but are not limited to, collagen, integrin, fibronectin, laminin, vitronectin, fibrinogen, an immunoglobulin superfamily member (e.g., CD2, CD4, CD8, ICAM1, ICAM2, VCAM1), selectin, cadherin, and the like. Most of these cell adhesion molecules transmit into a cell an auxiliary signal for cell activation due to intercellular interaction as well as cell adhesion. Therefore, an adhesion molecule for use in an implant of the present invention preferably transmits an auxiliary signal for cell activation into a cell. This is because cell activation can promote growth of cells originally present or aggregating in a tissue or organ at an injured site after application of an implant thereto. It can be determined whether or not such an auxiliary signal can be transmitted into a cell, by an assay, such as biochemical quantification (an SDS-PAGE method, a labeled-collagen method, etc.), immunological quantification (an enzyme antibody method, a fluorescent antibody method, an immunohistological study, etc.), a PCR method, a hybridization method, or the like, in which a positive reaction is detected.

An example of a cell adhesion molecule is cadherin which is present in many cells capable of being fixed to tissue. Cadherin can be used in a preferable embodiment of the present invention. Examples of a cell adhesion molecule in cells of blood and the immune system which are not fixed to tissue, include, but are not limited to, immunoglobulin superfamily molecules (LFA-3, CD2, CD4, CD8, ICAM-1, ICAM2, VCAM1, etc.); integrin family molecules (LFA-1, Mac-1, gpIIb/IIIa, p150, p95, VLA1, VLA2, VLA3, VLA4, VLA5, VLA6,

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etc.); selectin family molecules (L-selectin, E-selectin, P-selectin, etc.), and the like. Therefore, such a molecule may be useful for treatment of a tissue or organ of blood and the immune system.

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Nonfixed cells need to be adhered to a specific tissue in order to act on the tissue. In this case, it is believed that cell-cell adhesion is gradually enhanced via a first adhesion by a selectin molecule or the like which is constantly expressed and a second adhesion by a subsequently activated integrin molecule. Therefore, in the present invention, a cell adhesion molecule for mediating the first adhesion and another cell adhesion molecule for mediating the second adhesion may be used together.

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As used herein, the term "actin regulatory agent" refers to a substance which interacts directly or indirectly with actin in cells to change the form or state of the actin. It should be understood that actin regulatory agents are categorized into two classes, actin depolymerizing agents and actin polymerizing agents, depending on the action on actin. Examples of actin depolymerizing agents include, but are not limited to, Slingshot, cofilin, CAP (cyclase associated protein), ADF (actin depolymerizing factor), destrin, depactin, actophorin, cytochalasin, NGF (nerve growth factor), and the like. Examples of actin polymerizing agents include, but are not limited to, RhoA, mDi, profilin, Rac1, IRSp53, Wave2, profilin, ROCK, Lim kinase, cofilin, cdc42, N-WASP, Arp2/3, Drf3, IRSp53, Mena, LPA (lysophosphatidic acid), insulin, PDGF (platelet-derived growth factor) a, PDGFb, chemokine, TGF (transforming growth factor) b, and the like. The above-described actin regulatory agents include some substances which can be

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identified by the following assay. Interaction of an actin regulatory agent with respect to actin is assayed as follows. Actin is visualized using an actin staining reagent (Molecular Probes, Texas Red-X phalloidin) or the like. By
5 observing actin aggregation or cell outgrowth under a microscope, the presence of the interaction is determined by confirming the aggregation and reconstruction of actin and/or an increase in the cell outgrowth rate. The determination may be performed quantitatively or
10 qualitatively. The above-described actin regulatory agents are used in the present invention so as to promote the detachment or a multilayer structure of the synthetic tissue. When an actin regulatory agent used in the present invention is derived from an organism, the organism may be a mammalian
15 species, such as human, mouse, bovine, or the like.

The above-described agents involved in actin polymerization control actin polymerization in relation to Rho and the examples of the agents include the following (see,
20 for example, "Saibokokkaku/Undo ga wakaru (Understanding of cytoskeleton/movement)", (Ed./Hiroaki Miki), Yodo-sha).

Actin polymerization (see Takenaka T et al. J. Cell Sci., 114: 1801-1809, 2001)

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RhoA → mDi → profilin ⇒ actin polymerization

RhoA → ROCK/Rho → LIM kinase → phosphorylation of (suppression) ⇒ actin polymerization

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Rac1 → IRSp53 → WAVE2 → profilin, Arp2/3 ⇒ actin polymerization

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cdc42 → N-WASP → profilin, Arp2/3 ⇒ actin polymerization

cdc42 → Drf3 → IRSp53 → Mena ⇒ actin polymerization

5

(In the above descriptions, → indicates a signal transduction pathway such as phosphorylation. In the present invention any agent involved in such a pathway can be utilized.

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Actin depolymerization

Slingshot → dephosphorization of cofilin (activation) ⇒ actin depolymerization

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Actin depolymerization is controlled by a balance between phosphorylation by LIM kinase activity of cofilin and dephosphorization by Slingshot. As another agent for activating cofilin, CAP(cyclase-associated protein) and AIP1(actin-interacting-protein 1) are identified. It is recognized that any suitable agent can be used.

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LPA (lysophosphatidic acid) of any chain length can be used.

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Any chemokine can be used. However, examples of preferable chemokine include interleukin 8, MIP-1, SDF-1 and the like.

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Any TGFβ can be used. However, examples of preferable TGFβ include TGF-β1 and TGF-β3. TGF-β1 and TGF-β3 has an extracellular matrix generation promoting activity. Thus, in the present invention, TGF-β1 and TGF-β3 are used

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with an attention.

As used herein, the term "tissue strength" refers to a parameter which indicates a function of a tissue or organ and a physical strength of the tissue or organ. Tissue strength can be generally determined by measuring tensile strength (e.g., break strength, modulus of rigidity, Young's modulus, etc.). Such a general tensile test is well known. By analyzing data obtained by a general tensile test, various data, such as break strength, modulus of rigidity, Young's modulus, and the like, can be obtained. These values can be herein used as indicators of tissue strength. Typically, tissue strength which allows clinical applications is herein required.

The tensile strength of a synthetic tissue, three-dimensional structure, or the like of the present invention can be determined by measuring the stress and distortion characteristics thereof. Briefly, a load is applied to a sample; the resultant distortion and the load are input to respective A/D converters (e.g., ELK-5000) (1 ch: distortion, 2 ch: load); the stress and distortion characteristics are measured to determine the tensile strength of the sample (Figure 46). Tensile strength can also be determined by testing creep characteristics. A creep characteristics indentation test is conducted to investigate how a sample is extended over time while a constant load is applied to the sample. For small materials, thin materials, and the like, an indentation test is conducted using, for example, a triangular pyramid-shaped indenter with a tip having a radius of about 0.1 μm to about 1 μm . Initially, the indenter is pushed into a test piece so that a load is given to the test piece. When the indenter reaches from

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several tens of nanometers to several micrometers deep in the test piece, the indenter is drawn off to remove the load. Figure 47 shows a load/removal of load curve obtained by the above-described test method. Rigidity, Young's modulus, or the like can be obtained based on the behavior of the load and the push depth derived from the curve.

The tensile strength of the synthetic tissue of the present invention may be low. The tensile strength becomes higher when the matrix concentration is increased, and becomes lower when the cell to matrix ratio is increased. The present invention is characterized in that the strength can be adjusted as necessary. The present invention is also characterized in that the strength can be high or low relative to that of a tissue to be implanted. Therefore, it is recognized that the strength can be set to comply with any desired site.

As used herein, the term "physiologically active substance" refers to a substance capable of acting on a cell or tissue. Physiologically active substances include cytokines and growth factors. A cellular physiologically active substance may be naturally-occurring or synthesized. Preferably, a cellular physiologically active substance is one that is produced by a cell or one that has a function similar thereto. As used herein, a cellular physiologically active substance may be in the form of a protein or a nucleic acid or in other forms. In actual practice, cellular physiologically active substances are typically proteins. In the present invention, a physiologically active substance may be used to promote the affinity of an implanted synthetic tissue of the present invention, for example.

The term "cytokine" is used herein in the broadest sense in the art and refers to a physiologically active substance which is produced from a cell and acts on the same or different cell. Cytokines are generally proteins or polypeptides having a function of controlling an immune response, regulating the endocrine system, regulating the nervous system, acting against a tumor, acting against a virus, regulating cell growth, regulating cell differentiation, or the like. Cytokines are herein in the form of a protein or a nucleic acid or in other forms. In actual practice, cytokines are typically proteins.

The terms "growth factor" or "cell growth factor" are used herein interchangeably and each refers to a substance which promotes or controls cell growth. Growth factors are also called "proliferation factors" or "development factors". Growth factors may be added to cell or tissue culture medium, substituting for serum macromolecules. It has been revealed that a number of growth factors have a function of controlling differentiation in addition to a function of promoting cell growth.

Examples of cytokines representatively include, but are not limited to, interleukins, chemokines, hematopoietic factors such as colony stimulating factors, a tumor necrosis factor, interferons, a platelet-derived growth factor (PDGF), an epidermal growth factor (EGF), a fibroblast growth factor (FGF), a hepatocyte growth factor (HGF), a vascular endothelial cell growth factor (VEGF), cardiotrophin, and the like, which have proliferative activity.

Cellular physiologically active substances, such as cytokines, growth factors, and the like, typically have

redundancy in function. Accordingly, reference herein to a particular cytokine or growth factor by one name or function also includes any other names or functions by which the factor is known to those of skill in the art, as long as the factor has the activity of a cellular physiologically active substance for use in the present invention. Cytokines or growth factors can be used in a therapeutic or pharmaceutical agent according to a preferable embodiment of the present invention as long as they have preferable activity as described herein.

Therefore, in one embodiment of the present invention, it was revealed that when such a cytokine or growth factor (e.g., BMP-2, etc.) is provided to an implantation site (e.g., an injured site of a cartilage, etc.) concomitantly with a synthetic tissue or three-dimensional structure of the present invention, the affinity of the synthetic tissue or three-dimensional structure and an improvement in the function of the implantation site are observed. Thus, the present invention also provides such a combined therapy.

As used herein, the term "differentiation" refers to a developmental process of the state of the complex parts of organisms, such as cells, tissues, or organs and a process in which a characteristic tissue or organ is formed. The term "differentiation" is mainly used in embryology, developmental biology, and the like. In organisms, various tissues and organs are formed from divisions of a fertilized ovum (a single cell) to an adult. At early developmental stages (i.e., before cell division or after insufficient cell division), each cell or cell group has no morphological or functional feature and is not much distinguishable. Such a state is referred to as "undifferentiated".

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"Differentiation" may occur at the level of organs. A cell constituting an organ may develop into various cells or cell groups having different features. This phenomenon is also referred to as differentiation within an organ in the formation of the organ. Therefore, a synthetic tissue or three-dimensional structure of the present invention may comprise a tissue including differentiated cells.

When differentiation is required to produce a synthetic tissue of the present invention, the differentiation may be performed either before or after the organization of the cells.

As used herein, the terms "differentiation agent" and "differentiation promoting agent" are used interchangeably and refer to any agent which is known to promote differentiation of cells (e.g., chemical substances, temperature, etc.). Examples of such an agent include, but are not limited to, various environmental factors, such as temperature, humidity, pH, salt concentration, nutrients, metals, gas, organic solvent, pressure, chemical substances (e.g., steroids, antibiotics, etc.), and the like, or arbitrary combinations thereof. Representative examples of differentiation agents include, but are not limited to, cellular physiologically active substances. Representative examples of cellular physiologically active substances include, but are not limited to, DNA demethylating agents (e.g., 5-azacytidine, etc.), histone deacetylating agents (e.g., trichostatin, etc.), intranuclear receptor ligands (e.g., retinoic acid (ATRA), vitamin D₃, T₃, etc.), cell growth factors (e.g., activin, IGF-1, FGF, PDGF, TGF- β , BMP2/4, etc.), cytokines (e.g., LIF, IL-2, IL-6, etc.), hexamethylenebisacetamides, dimethylacetamides, dibutyl

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cAMPs, dimethylsulfoxides, iododeoxyuridines, hydroxyl ureas, cytosine arabinosides, mitomycin C, sodium lactate, aphidicolin, fluorodeoxyuridine, polybren hexadimetrine bromide, selenium, and the like.

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Specific examples of differentiation agents are described below. These differentiation agents may be used singly or in combination.

- 10 A) Cornea: epidermal growth factor (EGF);
- B) Skin (keratinocyte): TGF- β , FGF-7 (KGF: keratinocyte growth factor), EGF;
- C) Vascular endothelium: VEGF, FGF, angiopoietin;
- D) Kidney: LIF, BMP, FGF, GDNF;
- 15 E) Heart: HGF, LIF, VEGF;
- F) Liver: HGF, TGF- β , IL-6, EGF, VEGF;
- G) Umbilical endothelium: VEGF;
- H) Intestinal epithelium: EGF, IGF-1, HGF, KGF, TGF- β , IL-11;
- I) Nerve: nerve growth factor (NGF), BDNF (brain-derived neurotrophic factor), GDNF (glial-derived neurotrophic factor), neurotrophin, IL-6, TGF- β , TNF;
- 20 J) Glia cell: TGF- β , TNF- α , EGF, LIF, IL-6;
- K) Peripheral nerve cell: bFGF, LIF, TGF- β , IL-6, VEGF;
- L) Lung (alveolar epithelium): TGF- β , IL-13, IL-1 β , KGF, HGF;
- 25 M) Placenta: growth hormone (GH), IGF, prolactin, LIF, IL-1, activin A, EGF;
- N) Pancreatic epithelium: growth hormone, prolactin;
- O) Pancreatic Langerhans' cells: TGF- β , IGF, PDGF, EGF, TGF- β , TRH (thyrotropin);
- 30 P) Synovial cell: FGF, TGF- β (particularly, TGF- β 1, TGF- β 3);
- Q) Osteoblast: BMP (particularly, BMP-2, BMP-4, BMP-7), FGF;
- R) Chondroblast: FGF, TGF- β (particularly, TGF- β 1, TGF- β 3), BMP (particularly, BMP-2, BMP-4, BMP-7), TNF- α , IGF;

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- S) Retinal cell: FGF, CNTF (ciliary neurotrophic factor);
- T) Fat cell: insulin, IGF, LIF; and
- U) Muscle cell: LIF, TNF- α , FGF.

5 As used herein, the term "osteogenesis" indicates that any cell is caused to differentiate into an osteocyte. It is known that osteogenesis is promoted in the presence of dexamethasone, β -glycerophosphate, and ascorbic acid 2-phosphate. An osteogenic agent (BMP, (particularly, BMP-2, BMP-4, BMP-7)) may be added to promote osteogenesis.

10 As used herein, the term "chondrogenesis" refers to differentiation of any cell into a chondrocyte. It is known that chondrogenesis is promoted in the presence of pyruvic acid, dexamethasone, ascorbic acid 2-phosphate, insulin, transferrin, and selenious acid. An bone morphogenetic protein (BMP, (particularly, BMP-2, BMP-4, BMP-7)), TGF- β (particularly, TGF- β 1 and TGF- β 3), FGF, TNF- α and the like may be added to promote chondrogenesis.

20 As used herein, the term "adipogenesis" refers to differentiation of any cell into an adipocyte. It is known that adipogenesis is promoted in the presence of insulin, IGF, LIF, and ascorbic acid 2-phosphate.

25 As used herein, the terms "implant", "graft", and "tissue graft" are used interchangeably, referring to homologous or heterologous tissue or a cell group, or an artificial material, which is inserted into a particular site of a body and thereafter forms a part of the body. Therefore, a synthetic tissue or three-dimensional structure of the present invention can be used as an implant. Examples of conventional grafts include, but are not limited to, organs or portions of organs, blood vessels, blood vessel-like

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tissue, heart, cardiac valves, pericardia, duramatter, joint capsule, bone, cartilage, cornea, tooth, and the like. Therefore, grafts encompass anyone of these which is inserted into an injured part so as to compensate for the lost portion.

5 Grafts include, but are not limited to, autografts, allografts, and xenografts, which depend on the type of their donor.

As used herein, the term "autograft" (a tissue, a cell, an organ, etc.) refers to a graft (a tissue, a cell, an organ, etc.) which is implanted into the same individual from which the graft is derived. As used herein, the term "autograft" (a tissue, a cell, an organ, etc.) may encompass a graft from a genetically identical individual (e.g. an identical twin) in a broad sense. As used herein, the terms "autologous" and "derived from a subject" are used interchangeably. Therefore, the term "not derived from a subject" in relation to a graft indicates that the graft is not autologous (i.e., heterologous).

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As used herein, the term "allograft (a tissue, a cell, an organ, etc.)" refers to a graft (a tissue, a cell, an organ, etc.) which is transplanted from a donor genetically different from, though of the same species, as the recipient. Since an allograft is genetically different from the recipient, the allograft (a tissue, a cell, an organ, etc.) may elicit an immune reaction in the recipient. Examples of such grafts (a tissue, a cell, an organ, etc.) include, but are not limited to, grafts derived from parents (a tissue, a cell, an organ, etc.). The synthetic tissue of the present invention can be an allograft, which has been demonstrated to have satisfactory therapeutic results. Attention should be paid to the synthetic tissue of the present invention.

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As used herein, the term "xenograft" (a tissue, a cell, an organ, etc.) refers to a graft (a tissue, a cell, an organ, etc.) which is implanted from a different species. Therefore, for example, when a human is a recipient, a porcine-derived graft (a tissue, a cell, an organ, etc.) is called a xenograft (a tissue, a cell, an organ, etc.).

As used herein, "recipient" (acceptor) refers to an individual which receives a graft (a tissue, a cell, an organ, etc.) or implanted matter (a tissue, a cell, an organ, etc.) and is also called "host". In contrast, an individual providing a graft (a tissue, a cell, an organ, etc.) or implanted matter (a tissue, a cell, an organ, etc.) is called "donor" (provider).

With a synthetic tissue forming technique of the present invention, a synthetic tissue derived from any cell can be used. This is because a synthetic tissue (e.g., membranous tissues, organs, etc.) formed by the method of the present invention can exhibit a desired function while the tissue injury rate is maintained at a level which does not interfere with the therapy (i.e., a low level). Conventionally, tissues or organs are used as grafts without modification. In contrast to this, the present invention provides a tissue comprising three-dimensionally connected cells. Such a synthetic three-dimensional tissue cannot be achieved by conventional techniques, and therefore, constitutes one significant effect of the present invention.

As used herein, the term "subject" refers to an organism to which treatment of the present invention is applied and is also referred to as "patient". A patient or

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subject may be preferably a human.

Cells optionally used in a synthetic tissue, three-dimensional structure, or tissue graft of the present invention may be derived from a syngeneic origin (self origin), an allogenic origin (non-self origin), or a heterologous origin. In view of rejection reactions, syngeneic cells are preferable. If rejection reactions do not raise problems, allogenic cells may be employed. Cells which elicit rejection reactions can be employed by optionally treating the cells in a manner that overcomes rejection reactions. Procedures for avoiding rejection reactions are known in the art (see, for example, "Shin Gekagaku Taikai, Dai 12 Kan, Zoki Ishoku (Shinzo Ishoku · Hai Ishoku Gijyutsuteki, Rinriteki Seibi kara Jissai ni Mukete [New Whole Surgery, Vol. 12, Organ Transplantation (Heart Transplantation · Lung Transplantation From Technical and Ethical Improvements to Practice)" (Revised 3rd ed.), Nakayama Shoten]. Examples of such methods include, but are not limited to, a method using immunosuppressants or steroidal drugs, and the like. For example, there are currently the following immunosuppressants for preventing rejection reactions: "cyclosporine" (SANDIMMUNE/NEORAL); "tacrolimus" (PROGRAF); "azathioprine" (IMURAN); "steroid hormone" (prednisone, methylprednisone); and "T-cell antibodies" (OKT3, ATG, etc.). A method which is used worldwide as a preventive immunosuppression therapy in many facilities, is the concurrent use of three drugs: cyclosporine, azathioprine, and steroid hormones. An immunosuppressant is desirably administered concurrently with a pharmaceutical agent of the present invention. The present invention is not limited to this. An immunosuppressant may be administered before or after a regeneration/therapeutic method of the present

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invention as long as an immunosuppression effect can be achieved.

Cells used in the present invention may be derived
5 from any organism (e.g., vertebrates and invertebrates).
Preferably, cells derived from vertebrates are used. More
preferably, cells derived from mammals (e.g., primates,
rodents, etc.) are used. Even more preferably, cells derived
from primates are used. Most preferably, cells derived from
10 a human are used. Typically, cells from the same species
as the host are preferably used.

Examples of an affected portion of a subject treated
by a synthetic tissue of the present invention include, but
15 are not limited to, the heart suffering from a heart disease
(e.g., heart failure, ischemic heart diseases, myocardial
infarct, cardiomyopathy, myocarditis, hypertrophic
cardiomyopathy, dilated hypertrophic cardiomyopathy, and
dilated cardiomyopathy); blood vessels in a pericardium
patch, infarcted myocardium lower and upper limbs; a joint
20 injury or denaturation; a cartilage injury or denaturation;
osteonecrosis; meniscus injury or denaturation;
intervertebral disk denaturation; ligament injury or
denaturation; a fracture; implantation to a patient having
25 a joint, cartilage, or bone having bone loss; an injured
cornea; and the like.

Tissues targeted by the present invention may be any
organ of an organism and may be derived from any organism.
30 Examples of organisms targeted by the present invention
include vertebrates and invertebrates. Preferably,
organisms targeted by the present invention are mammals (e.g.,
primates, rodents, etc.). More preferably, organisms

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targeted by the present invention are primates. Most preferably, organisms targeted by the present invention are humans.

5 As used herein, the term "flexibility" in relation to a synthetic tissue refers to an ability to resist physical stimuli from external environments (e.g., pressure). A synthetic tissue having flexibility is preferable when the implantation site moves or deforms autonomously or by
10 external effects.

 As used herein, the term "extendibility and contractibility" in relation to a synthetic tissue refers to an ability to resist extending or contracting stimuli
15 from external environments (e.g., pulsation). A synthetic tissue having extendibility and contractibility is preferable when the implantation site is subjected to extending or contracting stimuli. Examples of implantation sites, which are subjected to extending or contracting
20 stimuli, include, but are not limited to, heart, muscle, joint, cartilage, tendon, and the like. In one embodiment, extendibility and contractibility capable of withstanding the pulsation motion of the heart may be required.

25 As used herein, the term "part" or "portion" refers to any part or portion, tissue, cell, or organ in the body. Examples of such parts, tissues, cells, and organs include, but are not limited to, a portion which can be treated with skeletal myoblasts, fibroblasts, synovial cells, stem cells,
30 and the like. A marker specific to a portion may be any parameter, such as a nucleic acid molecule (expression of mRNA), a protein, an extracellular matrix, a specific phenotype, a specific shape of a cell, or the like. Therefore,

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markers which are not specified herein may be used to identify a synthetic tissue of the present invention as long as these markers can indicate cells derived from a portion. Representative examples of portions, but are not limited to, portions of the heart other than the adult myocardium, portions containing mesenchymal stem cells or cells derived therefrom, other tissues, other organs, myoblasts (e.g., skeletal myoblasts), fibroblasts, synovial cells, and the like.

For observing a cartilage tissue, following markers can be used as index.

Sox9 (human: Accession No. NM_000346) is a marker specific to chondrocyte. The marker can be confirmed mainly by observing the presence of mRNA (Kulyk WM, Franklin JL, Hoffman LM. Sox9 expression during chondrogenesis in micromass cultures of embryonic limb mesenchyme. Exp Cell Res. 2000 Mar 15; 255(2):327-32.).

Col 2A1 (human: Accession No. NM_001844) is a marker specific to chondrocyte. The marker can be confirmed mainly by observing the presence of mRNA (Kulyk WM, Franklin JL, Hoffman LM. Sox9 expression during chondrogenesis in micromass cultures of embryonic limb mesenchyme. Exp Cell Res. 2000 Mar 15; 255(2):327-32.).

Aggrecan (human: Accession No. NM_001135) is a marker specific to chondrocyte. The marker can be confirmed mainly by observing the presence of mRNA (Kulyk WM, Franklin JL, Hoffman LM. Sox9 expression during chondrogenesis in micromass cultures of embryonic limb mesenchyme. Exp Cell Res. 2000 Mar 15; 255(2):327-32.).

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Bone sialoprotein (human: Accession No. NM_004967) is a marker specific to an osteoblast. The marker can be confirmed mainly by observing the presence of mRNA (Haase HR, Ivanovski S, Waters MJ, Bartold PM. Growth hormone regulates osteogenic marker mRNA expression in human periodontal fibroblasts and alveolar bone-derived cells. J Periodontal Res. 2003 Aug;38(4):366-74.).

10 Osteocalcin (human: Accession No. NM_199173) is a marker specific to an osteoblast. The marker can be confirmed mainly by observing the presence of mRNA (Haase HR, Ivanovski S, Waters MJ, Bartold PM. Growth hormone regulates osteogenic marker mRNA expression in human periodontal
15 fibroblasts and alveolar bone-derived cells. J Periodontal Res. 2003 Aug;38(4):366-74.).

GDF5 (human :Accession No. NM_000557) is a marker specific to a ligament cell. The marker can be confirmed
20 mainly by observing the presence of mRNA (Wolfman NM, Hatteraley G, Cox K, Celeste AJ, Nelson R, Yamaji N, Dube JL, DiBlasio-Smith E, Nove J, Song JJ, Wozney JM, Rosen V. Ectopic induction of tendon and ligament in rats by growth and differentiation factors 5, 6, and 7, members of the
25 TGF-beta gene family. J Clin Invest. 1997 Jul 15;100(2):321-30.).

30 Six1 (human: Accession No. NM_005982) is a marker specific to a ligament cell (Drayer SD, Naruse T, Morello R, Zabel B, Winterpacht A, Johnson RL, Lee B, Oberg KC. Ix1b expression during joint and tendon formation: localization and evaluation of potential downstream targets. Gene Expr Patterns. 2004 Jul;4(4):397-405.). The marker can be

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confirmed mainly by observing the presence of mRNA.

Scieraxis (human :Accession No. BK000280) is a marker specific to a ligament cell (Brent AE, Schweitzer R, Tabin CJ. A somitic compartment of tendon progenitors. Cell. 2003 Apr 18;113(2):235-48.). The marker can be confirmed mainly by observing the presence of mRNA.

A "part other than the myocardium of an adult" and a "part other than the heart of an adult" can be identified using markers characteristic to cells derived from the myocardium of an adult or the heart of an adult including skeletal myoblasts, fibroblasts, synovial cells, stem cells, or the like (hereinafter referred to as a "non-adult myocardial marker" or a "non-adult heart marker", respectively). If the marker is expressed by less than about 100%, preferably less than about 80%, more preferably less than about 50%, even more preferably less than about 25%, in some cases less than about 1%, the above-described parts can be identified. Examples of such markers include, but are not limited to, myosin heavy chain IIa, myosin heavy chain IIb, myosin heavy chain IIc (IIx), CD56, MyoD, Myf5, myogenin, and the like. Therefore, non-adult myocardial markers which are not specified herein may be used to identify a synthetic tissue of the present invention as long as these markers can indicate cells derived from parts other than the myocardium of an adult. Also, non-adult heart markers which are not specified herein may be used to identify a synthetic tissue of the present invention as long as these markers can indicate cells derived from parts other than the heart of an adult.

Myosin heavy chain IIa (human: Accession

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No. NM_017534; SEQ ID NOs. 1 and 2), myosin heavy chain IIb (human: Accession No. NM_017533; SEQ ID NOs. 3 and 4), and myosin heavy chain IIc (IIx) (human: Accession No. NM_005963; SEQ ID NOs. 5 and 6) are markers specific to myoblasts (Havenith M.G., Visser R., Schrijvers-van Schendel J.M., Bosman F.T., "Muscle Fiber Typing in Routinely Processed Skeletal Muscle With Monoclonal Antibodies", Histochemistry, 1990; 93(5):497-499). These markers can be confirmed mainly by observing the presence of proteins. An antibody against myosin heavy chain IIa, myosin heavy chain IIb, and myosin heavy chain IIc (IIx) is, for example, MY-32 available from Sigma. This antibody is specific to skeletal muscles and does not bind to myocardium (Webster C., Pavlath G.K., Parks D.R., Walsh F.S., Blau N.M., Exp. Cell. Res., 1988 Jan; 174(1):252-65; and Havenith M.G., Visser R., Schrijvers-van Schendel J.M., Bosman F.T., Muscle Fiber Typing in Routinely Processed Skeletal Muscle with Monoclonal Antibodies, Histochemistry, 1990, 93(5):497-499).

CD56 (human: Accession No. U63041; SEQ ID NOs. 7 and 8) is a marker specific to myoblasts. This marker can be confirmed mainly by observing the presence of mRNA.

MyoD (human: Accession No. X56677; SEQ ID NOs. 9 and 10) is a marker specific to myoblasts. This marker can be confirmed mainly by observing the presence of mRNA.

Myf5 (human: Accession No. NM_005593; SEQ ID NOs. 11 and 12) is a marker specific to myoblasts. This marker can be confirmed mainly by observing the presence of mRNA.

Myogenin (human: Accession No. BT007233; SEQ ID NOs. 13 and 14) is a marker specific to myoblasts. This

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marker can be confirmed mainly by observing the presence of mRNA.

5 In other embodiments, other markers specific to other tissues can be utilized. Examples of such markers include, but are not limited to, Oct-3/4, SSEA-1, Rex-1, Otx2, and the like for embryonic stem cells; VE-cadherin, Flk-1, Tie-1, PECAM1, vWF, c-kit, CD34, Thyl, Sca-1, and the like for endothelial cells; skeletal muscle α actin in addition to
10 the above-described markers for skeletal muscles; Nestin, Glu receptor, NMDA receptor, GFAP, neuregulin-1, and the like for nerve cells; c-kit, CD34, Thyl, Sca-1, GATA-1, GATA-2, PDG, and the like for hematopoietic cells.

15 As used herein, the term "derived" in relation to cells means that the cells are separated, isolated, or extracted from a cell mass, tissue, or organ in which the cells have been originally present, or that the cells are induced from stem cells.

20 As used herein, the term "applicable to heart" means that the heart applied has an ability to pulsate. A tissue applicable to heart has strength such that the tissue can withstand dilation and contraction of the pulsating heart.
25 Here, applicability to the heart includes applicability to the myocardium. Applicability to heart may be determined by confirming that a recipient having an implanted graft survives.

30 As used herein, the term "three-dimensional structure" refers to an object which comprises cells having intracellular intergration or alignment and extends three-dimensionally, particularly matrices are oriented

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three-dimensionally and cells are arranged three-dimensionally.

As used herein, the term "biological integration" in relation to the relationship between biological entities such as cells means that there is certain interaction between the biological entities. Examples of such interaction includes, but are not limited to, interaction via biological molecules (e.g., extracellular matrix), interaction via signal transduction, electrical interaction (electrical integration, such as synchronization of electrical signals or the like), and the like. Biological integration includes biological integration in a synthetic tissue and biological integration of a synthetic tissue with its surroundings (e.g., surrounding tissues and cells after implantation, etc.). In order to confirm interactions, an assay appropriate to a characteristic of the interaction is employed. In order to confirm physical interactions via biological molecules, the strength of a synthetic tissue, a three-dimensional structure, or the like is measured (e.g., a tensile test). In order to confirm interaction via signal transduction, gene expression or the like is investigated. In order to confirm electrical interactions, the electric potential of a synthetic tissue, a three-dimensional structure, or the like is measured to determine whether or not the electric potential is propagated with constant waves. In the present invention, biological integration is provided in all three dimensions. Preferably, there is biological integration substantially uniformly in all directions in a three-dimensional space. In another embodiment, the synthetic tissue, a three-dimensional structure, and the like, which has substantially uniform two-dimensional biological integration and slightly weaker biological

integration in the third dimension, may be employed. Biological integration via an extracellular matrix can be confirmed based on the degree of staining by staining the extracellular matrix. As a method for observing biological
5 integration *in vivo*, there is an integration experiment using cartilage. In this experiment, a surface of the cartilage is removed and digested with chondroitinase ABC (Hunziker E.B. et al., J. Bone Joint Surg. Am., 1996 May; 78(5): 721-33). Thereafter, a tissue of interest is implanted onto a cut
10 surface, followed by culturing for about 7 days. The subsequent integration is observed (Figure 23). It will be understood that a capability to adhere to surrounding cells can be determined with the above-described cartilage experiment.

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A synthetic tissue, three-dimensional structure, or the like of the present invention may be provided using known preparation methods, as a pharmaceutical product, or alternatively, as an animal drug, a quasi-drug, a marine
20 drug, a cosmetic product, and the like.

Animals targeted by the present invention include any organism as long as it has organs (e.g., animals (e.g., vertebrates, invertebrate)). Preferably, the animal is a
25 vertebrate (e.g., Myxiniiformes, Petromyzoniformes, Chondrichthyes, Osteichthyes, amphibian, reptilian, avian, mammalian, etc.), more preferably mammalian (e.g., monotremata, marsupialia, edentate, dermoptera, chiroptera, carnivore, insectivore, proboscidea, perissodactyla,
30 artiodactyla, tubulidentata, pholidota, sirenia, cetacean, primates, rodentia, lagomorpha, etc.). Illustrative examples of a subject include, but are not limited to, animals, such as cattle, pigs, horses, chickens, cats, dogs, and the

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like. More preferably, primates (e.g., chimpanzee, Japanese monkey, human, etc.) are used. Most preferably, a human is used. This is because there is limitation to implantation therapies.

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When the present invention is used as a pharmaceutical agent, it may further comprise a pharmaceutically acceptable carrier or the like. A pharmaceutically acceptable carrier contained in a medicament of the present invention includes
10 any material known in the art.

Examples of such a pharmaceutically acceptable carrier include, but are not limited to, antioxidants, preservatives, colorants, flavoring agents, diluents,
15 emulsifiers, suspending agents, solvents, fillers, bulking agents, buffers, delivery vehicles, agricultural or pharmaceutical adjuvants, and the like.

The amount of a pharmaceutical agent (e.g., a synthetic tissue, a pharmaceutical compound used in conjunction therewith, etc.) used in the treatment method of the present invention can be easily determined by those skilled in the art with reference to the purpose of use, a target disease (type, severity, and the like), the patient's
20 age, weight, sex, and case history, the form or type of the cell, and the like. The frequency of the treatment method of the present invention applied to a subject (or patient) is also determined by those skilled in the art with respect to the purpose of use, target disease (type, severity, and
25 the like), the patient's age, weight, sex, and case history, the progression of the therapy, and the like. Examples of the frequency include once per day to several months (e.g., once per week to once per month). Preferably, administration
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is performed once per week to month with reference to the progression.

As used herein, the term "administer" in relation to a synthetic tissue, three-dimensional structure, or the like of the present invention or a pharmaceutical agent comprising it, means that they are administered singly or in combination with other therapeutic agents. A synthetic tissue of the present invention may be introduced into therapy sites (e.g., impaired heart, etc.) by the following methods, in the following forms, and in the following amounts. Examples of the introduction methods include, but are not limited to, direct attachment, suture after attachment, insertion, and the like. For example, a synthetic tissue and a three-dimensional structure of the present invention may be applied by the above-described methods to an impaired site of ischemic myocardial tissue caused by myocardial infarct, angina pectoris, or the like. Combinations may be administered either concomitantly (e.g., as an admixture), separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously (e.g., a synthetic tissue or the like is directly provided by operation, while other pharmaceutical agents are provided by intravenous injection). "Combination" administration further includes the separate administration of one of the compounds or agents given first, followed by the second.

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As used herein, the term "reinforcement" means that the function of a targeted part of an organism is improved.

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As used herein, the term "instructions" describe how to handle reagents, usage, a preparation method, a method of producing a synthetic tissue, a method of administering a medicament of the present invention, a method for diagnosis, or the like for persons who administer, or are administered, the medicament or the like or persons who diagnose or are diagnosed (e.g., physicians, patients, and the like). The instructions describe a statement indicating an appropriate method for administering a diagnostic, a medicament, or the like of the present invention. The instructions are prepared in accordance with a format defined by an authority of a country in which the present invention is practiced (e.g., Health, Labor and Welfare Ministry in Japan, Food and Drug Administration (FDA) in the U.S., and the like), explicitly describing that the instructions are approved by the authority. The instructions are so-called package insert and are typically provided in paper media. The instructions are not so limited and may be provided in the form of electronic media (e.g., web sites, electronic mails, and the like provided on the Internet).

As used herein, the term "extracellular matrix synthesis promoting agent" or "ECM synthesis promoting agent" refers to an agent which promotes the production of an extracellular matrix of a cell. In the present invention, when an ECM synthesis promoting agent is added to a cell sheet, an environment which promotes self-contraction of cells after a cell sheet is detached from a culture container. The sheet is biologically organized in three-dimensional directions. Examples of such an agent representatively include agents capable of promoting the secretion of an extracellular matrix (e.g., TGF- β 1, TGF- β 3, etc.). Examples of an ECM synthesis promoting agent representatively include,

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but are not limited to, TGF- β 1, TGF- β 3, ascorbic acid, ascorbic acid 2-phosphate, or a derivative or salt thereof. Preferably, an ECM synthesis promoting agent may be preferably a component of an extracellular matrix of a part
5 targeted by application and/or a component(s) capable of promoting the secretion of an extracellular matrix in an amount similar thereto. When an ECM synthesis promoting agent comprises a plurality of components, the components may be components of an extracellular matrix of a part targeted
10 by application and/or components capable of promoting the secretion of an extracellular matrix in an amount similar thereto.

As used herein, the term "ascorbic acid or a
15 derivative thereof" includes ascorbic acid and an analog thereto (e.g., ascorbic acid 2-phosphate, ascorbic acid 1-phosphate, etc.), and a salt thereof (e.g., sodium salt, magnesium salt, etc.). Ascorbic acid is preferably, but is not limited to, an L-isomer.

20

(Description of the Preferred Embodiments)

Hereinafter, preferable embodiments of the present invention will be described. The following embodiments are provided for a better understanding of the present invention
25 and the scope of the present invention should not be limited to the following description. It will be clearly appreciated by those skilled in the art that variations and modifications can be made without departing from the scope of the present invention with reference to the specification.

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In an aspect of the present invention, the synthetic tissue and complex of the present invention is free of injury caused by a protein degrading enzyme, such as,

representatively, dispase, trypsin, or the like, during culture. Therefore, the synthetic tissue and complex, which is detached from the base material, can be recovered as a cell mass holding proteins between cells (e.g., an extracellular matrix) and having a certain level of strength. The synthetic tissue and complex also retain intact functions, such as an intracellular linking manner, alignment, and the like. When typical protein-degrading enzymes (e.g., trypsin, etc.) are used to detach the three-dimensional structure or synthetic tissue, substantially no cell-to-cell link or cell-to-extracellular matrix link are retained, so that cells are individually separated. Among these protein-degrading enzymes, dispase destroys basement membrane-like proteins between cells and base materials substantially completely. In this case, however, the resultant three-dimensional structure or synthetic tissue has weak strength. In contrast, the three-dimensional structure or synthetic tissue of the present invention can both substantially completely retain each of the desmosome structure and the basement membrane-like protein, resulting in the above-described various effects.

In the method of the present invention, the period of time required for culture may be determined depending on the application of the synthetic tissue or three-dimensional structure. In order to detach and recover the cultured synthetic tissue or three-dimensional structure from the support material, the cultured synthetic tissue or three-dimensional structure is detached directly, or with macromolecular membrane being attached thereto. Note that the synthetic tissue or three-dimensional structure may be detached in culture medium in which cells have been cultured, or alternatively, in other isotonic solutions. Such

solutions may be selected depending on the purpose. When a monolayer cell sheet is prepared, examples of the macromolecular membrane, which is optionally attached to the cell sheet or three-dimensional structure, include, but are not limited to, hydrophilized polyvinylidene difluoride (PVDF), polypropylene, polyethylene, cellulose and derivatives thereof, chitin, chitosan, collagen, paper (e.g., Japan paper, etc.), urethane, net-like or stockinette-like macromolecular materials (e.g., spandex, etc.), and the like.

When a net-like or stockinette-like macromolecular material is employed, the synthetic tissue or complex has a higher degree of freedom, so that the contraction/relaxation function thereof can be increased. A method for producing the synthetic tissue or three-dimensional structure comprising cells of the present invention is not particularly limited. For example, the synthetic tissue or three-dimensional structure of the present invention can be produced by utilizing the above-described cultured cell sheet attached to a macromolecular membrane.

In order to detach and recover the synthetic tissue or complex with a high yield from the cell culture support, the cell culture support is tapped or shaken, or the medium is stirred with a pipette. These procedures may be performed singly or in combination. In addition, the synthetic tissue or complex may be detached and recovered by deforming the base of the culture container or rinsing the container with isotonic solution or the like. By stretching the synthetic tissue or complex in a specific direction after being detached from the base material, the complex is provided with alignment. Stretching may be performed by using a tensile device (e.g., Tensilon, etc.), or simply forceps, or the like. A stretching method is not particularly limited. By providing alignment,

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it is possible to confer directionality to the motion of the cell sheet or complex itself. Therefore, for example, it is possible to allow the synthetic tissue or complex to move in accordance with the motion of a specific organ. The synthetic tissue or complex can be efficiently applied to organs.

The thus-obtained synthetic tissue or complex cannot be obtained by conventional techniques.

The synthetic tissue and the complex according to the present invention includes an abundance of adhesion molecules such as extracellular matrix which may include collagen (types I, III, etc.), vitronectin, and fibronectin, and can be accepted by the surrounding tissue. Thus, implanted cells can be stably accepted by the implantation site. In conventional cell implantation, it was difficult for cells to be stably accepted by the implantation site not only in cell implantation without a scaffold, but also in cell implantation using an additional stabilizing treatment (e.g., sewing of a patch, scaffold, etc.). However, use of the present invention facilitates stabilization. When only cells are used, reinforcement by another tissue, fixing scaffold, or the like is necessary. According to the present invention, without requiring such means, cells which may have pluripotency included in the synthetic tissue or complex can be stably accepted by the implantation portion without an additional fixing means.

(Preparation of synthetic tissue using an ECM synthesis promoting agent)

In another aspect, the present invention provides a method for producing a synthetic tissue. The method for

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producing a synthetic tissue comprises the steps of:
A) providing a cell; B) placing the cell in a container
containing a cell culture medium including an ECM synthesis
promoting agent, wherein the container has a base with an
5 area sufficient to accommodate a desired size of the synthetic
tissue; and C) culturing the cell in the container for a
period of time sufficient to form the synthetic tissue having
the desired size.

10 The above-described cell may be any cell. A method
for providing a cell is well known in the art. For example,
a tissue is extracted and cells are isolated from the tissue.
Alternatively, cells are isolated from body fluid containing
blood cells or the like. Alternatively, a cell line is
15 prepared in an artificial culture. The present invention
is not limited to this. Cells used herein may be any stem
cells or differentiated cells, particularly including
myoblasts, mesenchymal stem cells, adipocytes, synovial
cells, bone marrow cells, and the like. Examples of
20 mesenchymal stem cells used herein include adipose
tissue-derived stem cells, bone marrow-derived stem cells,
and the like.

25 The method for producing a synthetic tissue of the
present invention employs a cell culture medium containing
an ECM synthesis promoting agent. Examples of such an ECM
synthesis promoting agent include, but are not limited to,
ascorbic acid or a derivative thereof, ascorbic acid
1-phosphate, ascorbic acid 2-phosphate, L-ascorbic acid,
30 and the like.

The cell culture medium used in the present invention
may be any medium which allows a cell of interest to grow.

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Examples of such a medium include, but are not limited to, DMEM, MEM, F12, DME, RPMI1640, MCDB104, 199, MCDB153, L15, SkEM, Basal medium, and the like which are supplemented with glucose, FCS (fetal calf serum), antibiotics (penicillin, streptomycin, etc.) as appropriate.

The container used in the present invention may be any container typically used in the art which has a base with an area sufficient to accommodate a desired size of the synthetic tissue. Examples of such a container include, but are not limited to, petridishes, flasks, mold containers, and the like, and preferably containers having a large area of the base (e.g., at least 1 cm²). The material of the container may be any material and include, but are not limited to, glass, plastic (e.g., polystyrene, polycarbonate, etc.), silicone, and the like.

In a preferable embodiment, the method for producing a synthetic tissue according to the present invention further comprises detaching a produced synthetic tissue. As used herein, the term "detach" indicates that after a synthetic tissue of the present invention is formed in a container, the synthetic tissue is removed from the container. The detachment can be achieved by, for example, physical means (e.g., pipetting of medium, etc.), chemical means (addition of a substance), or the like. In the present invention, a synthetic tissue can be detached by providing a stimulus around the synthetic tissue by physical means or chemical means, but not by aggressive means (e.g., treatment with a protein degrading enzyme, etc.) to the synthetic tissue. Thus, the present invention provides ease of handling, which cannot be conventionally achieved, and the resulting synthetic tissue is substantially intact, resulting in a

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high-performance implant.

In a preferable embodiment, the present invention further comprises detaching cells which construct a synthetic tissue. In a more preferable embodiment, the detaching step includes applying a stimulus for contracting a synthetic tissue, including a physical stimulus (e.g., pipetting, etc.). Such a physical stimulus is not directly applied to the produced synthetic tissue. This is a preferable feature of the present invention. Since a physical stimulus is not directly applied to a synthetic tissue, it is possible to suppress damage to the synthetic tissue. Alternatively, the detaching step includes chemical means, such as adding an actin regulatory agent. Such an actin regulatory agent includes a chemical substance selected from the group consisting of actin depolymerizing agents and actin polymerizing agents. Examples of actin depolymerizing agents include, but are not limited to, ADF (actin depolymerizing factor), destrin, depactin, actophorin, cytochalasin, NGF (nerve growth factor), and the like. Examples of actin polymerizing agents include, but are not limited to, LPA (lysophosphatidic acid), insulin, PDGF α , chemokine, TGF β , and the like.

Though not wishing to be bound by any theory, these actin regulatory agents may cause actomyosin-based cytoskeleton to contract or extend, thereby regulating contraction and extension of a cell itself. As a result, a synthetic tissue itself may be promoted to or inhibited from being detached from the base of a container.

In another embodiment, the synthetic tissue and complex of the present invention are characterized in that

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they are produced from cells which are cultured in monolayer culture. Despite monolayer culture, synthetic tissues having various thicknesses can be constructed. This is an unexpected effect. Conventionally, for example, a thick
5 tissue cannot be constructed without using a multilayer structure when a temperature responsive sheet or the like is used. The present invention is the first to achieve a method for constructing a three-dimensional structure, which does not require a scaffold and can construct the contractile
10 organization including ten or more layers. A typical cell implantation method which does not employ a scaffold is a cell sheet engineering technique utilizing a temperature sensitive culture dish disclosed by Kushida A., Yamato M., Konno C., Kikuchi A., Sakurai Y., Okano T., *J. Biomed. Mater. Res.*, 45:355-362, 1999. The technique has won international
15 recognition as an original technique. However, this cell sheet technique has a problem in that a single sheet is weak in many cases, and requires modification such as layering sheets for obtaining the strength resistant to an surgical operation such as implantation.
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A cell/matrix complex developed by the present invention does not require a temperature sensitive culture dish unlike the cell sheet technique. The cell/matrix
25 complex is easy to form into a contractile three-dimensional tissue. There is no technique in the world other than the present invention, which can produce a contractile three-dimensional complex having 10 or more layers without using so-called feeder cells, such as rodent stroma cells,
30 after approximately three weeks. By adjusting conditions for matrix production of the synovial cell, it is possible to produce a complex having a strength which allows surgical manipulation, such as holding or transferring the complex,

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without a special instrument. Therefore, the present invention is an original, epoch-making technique in the world for reliably and safely perform cell implantation.

5 In a preferable embodiment, the ECM synthesis promoting agent used in the method for producing a synthetic tissue of the present invention includes ascorbic acid 2-phosphate (Hata R., Senoo H., J. Cell Physiol., 1989, 138(1):8-16). In the present invention, by adding a certain
10 amount or more of ascorbic acid 2-phosphate, it is possible to promote production of an extracellular matrix, so that the resultant synthetic tissue or complex is made strong to become easy to be detached. Thereafter, self contraction is elicited by applying a stimulus for detachment. Hata et al.
15 do not report that, after adding such an ascorbic acid and culturing, a tissue becomes strong and obtains a property to be easy to be detached. Though not wishing to be bound by any theory, a significant difference is that Hata et al. used a significantly different cell density. Hata et al.
20 does not suggest an effect of making a tissue rigid. Such an effect that the tissue is made rigid, an effect of contraction, and an effect that the tissue becomes easy to be detached are first found in the present invention. The synthetic tissue according to the present invention is
25 recognized to be totally different from the synthetic tissue which has been fabricated conventionally at least on the point that it is produced through the process of making rigid, contraction, and detachment.

30 Contraction when the culture is detached and promotion in constructing a three-dimensional structure, a contractile three-dimensional tissue, or the like are surprising effects. Such effects have not been reported conventionally.

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In a preferable embodiment, ascorbic acid 2-phosphate used in the present invention typically has a concentration of at least 0.01 mM, preferably at least 0.05 mM, more preferably at least 0.1 mM, even more preferably at least 0.2 mM, still more preferably at least 0.5 mM, and still even more preferably 1.0 mM. Herein, any concentration of 0.1 mM or higher may be employed. However, there may be an aspect in which a concentration of 10 mM or lower is desired.

In a certain preferable embodiment, the ECM synthesis promoting agent of the present invention includes ascorbic acid 2-phosphate or a salt thereof, and L-ascorbic acid or a salt thereof.

In a preferable embodiment, after the culturing step, the synthetic tissue production method of the present invention further comprises, detaching the synthetic tissue and allowing the synthetic tissue to perform self contraction. The detachment can be accelerated by applying a physical stimulus (e.g., application of shear stress, pipetting, deformation of the container, etc.). Self-contraction naturally takes place when a stimulus is applied after the detachment. When a chemical stimulus is applied, self-contraction and detachment occurs simultaneously. By self-contraction, biological integration is accelerated particularly in the third dimension (the direction perpendicular to the two-dimensional directions in the case of tissue on a sheet). Therefore, a synthetic tissue of the present invention may have a three-dimensional structure.

In a synthetic tissue production method of the present

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invention, the sufficient time preferably means at least 3 days, though it varies depending on the application of a synthetic tissue of interest. An exemplary period of time is 3 to 7 days.

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In another embodiment, the synthetic tissue production method of the present invention may further comprise causing a synthetic tissue to differentiate. By differentiation, the synthetic tissue can have a form closer
10 to that of a desired tissue. An example of such differentiation is, but is not limited to, chondrogenesis and osteogenesis. In a preferable embodiment, osteogenesis may be performed in medium containing dexamethasone, β -glycerophosphate, and ascorbic acid 2-phosphate. More
15 preferably, bone morphogenetic proteins (BMPs) are added. This is because such BMP-2, BMP-4, and BMP-7 proteins promote osteogenesis.

In another embodiment, a method of producing the
20 synthetic tissue of the present invention is a process of differentiating a synthetic tissue. A form of differentiation includes performing a differentiation of cartilage. In the preferable embodiment, chondrogenesis is performed in a medium including pyruvic acid, dexamethasone,
25 ascorbic acid 2-phosphate, insulin, transferrin, and selenious acid. More preferably, bone morphogenetic proteins (such as BMP-2, BMP-4, BMP-7), transforming growth factors (such as TGF- β 1, TGF- β 3) are added. This is because such BMPs promote chondrogenesis.

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An important point in the present invention is that it is possible to fabricate a tissue having a pluripotency into various differentiated cells such as bone, cartilage,

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and the like. Conventionally, differentiation into a cartilage tissue is difficult in other synthetic tissues which are scaffold-free. If a certain size is required, conventionally, it was necessary to coculture with a scaffold, construct a three-dimensional structure, and add a chondrogenesis medium. Conventionally, scaffold-free differentiation into cartilage was difficult. The present invention is the first to enable differentiation into cartilage in a synthetic tissue. This is not an effect which has not been obtained conventionally, and is a characteristic effect of the present invention. In a treatment which aims to regenerate a tissue, a method for performing a treatment efficiently and safely by using a tissue of sufficient size without a scaffold was difficult. The present invention achieves a significant effect on this point. Particularly, the present invention is significant on the point that it becomes possible to easily manipulate differentiated cells such as cartilage, which has been impossible conventionally. Conventionally, for example, cells can be collected to a pellet shape and the aggregation of cells can be differentiated to obtain a tissue of about 2 mm³. For obtaining a tissue larger than this size, it was necessary to use a scaffold.

The differentiation step in synthetic tissue production of the present invention may be performed before or after providing cells.

In the present invention, primary culture cells can be used. The present invention is not limited to this. Subcultured cells (e.g., three or more passages) can also be used. Preferably, when subculture cells are used, the cells are preferably of four passages or more, more preferably

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of 5 passages or more, and even more preferably of 6 passages or more. The upper limit of cell density is increased with an increase in the number of passages within a certain range. This is because a denser synthetic tissue can be produced.

5 The present invention is not limited to this. It seems that a certain range of passages (e.g., 3 to 8 passages) are preferable.

In the present invention, the cells are preferably provided at a cell density of $5.0 \times 10^4 / \text{cm}^2$ or more. The present invention is not limited to this. This is because a higher cell density can provide a synthetic tissue having a greater strength. It will be understood that the lower limit of the cell density may be lower than the above-described density.

10

15 It will also be understood that those skilled in the art can define the lower limit based on the present specification.

In one embodiment of the present invention, for example, a myoblast, a synovial cell, an adipocyte, and a mesenchymal stem cell (e.g., derived from adipose tissue or bone marrow) can be used. The present invention is not limited to this. These cells can be applied to, for example, a heart, a bone, a cartilage, a tendon, a ligament, a joint, a meniscus, and the like.

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25

(Synthetic tissue and complex)

In another aspect, the present invention provides a functional synthetic tissue or complex. The functional synthetic tissue of the present invention is herein an implantable synthetic tissue. Attempts have been heretofore made to produce synthetic tissues by cell culture. However, there were no synthetic tissues suitable for implantation in terms of size, strength, physical injuries

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when it is detached from a culture container, or the like. The present invention provides a tissue culture method in which cells are cultured in the presence of an ECM synthesis promoting agent as described above, so that there is no problem in terms of size, strength, and the like and there is no difficulty in detaching tissues. An implantable synthetic tissue is provided only after such a tissue culture method is achieved.

Another aspect of the present invention provides cells, and a complex including factors derived from the cells. Herein, it is recognized that, preferably, the complex substantially comprises cells, and the factors derived from the cells. Herein, the complex of the present invention is provided for reinforcing, repairing, or regenerating a part of an organism.

As used herein, the term "complex" means that cells and other components are integrated into a complex by some kind of interactivity. Therefore, the complex of the present invention often has an appearance like a synthetic tissue, and it is recognized that the meaning of the term "complex" overlaps with what is referred to by a synthetic tissue.

The present invention provides a scaffold-free synthetic tissue or complex. A therapeutic method and a therapeutic agent for providing an excellent condition after implantation can be obtained by providing such a scaffold-free synthetic tissue.

The scaffold-free synthetic tissue of the present invention solves a long outstanding problem with biological formulations, which is attributed to contamination of the

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scaffold itself. Despite the absence of a scaffold, the therapeutic effect is comparable with, or more satisfactory than, conventional techniques.

5 In addition, when a scaffold is used, the alignment of implanted cells in the scaffold, the cell-to-cell adhesion, the in vivo alteration of the scaffold itself (eliciting inflammation), the acceptance of the scaffold by the recipient tissue, and the like become problematic.
10 These problems can be solved by the present invention.

 The synthetic tissue and the complex of the present invention are also self-organized, and have biological integration inside thereof. Also in this point, the present
15 invention is distinguished from conventional cell therapies.

 The synthetic tissue and the complex of the present invention are easily used to form a three-dimensional structure, and is thus easy to be designed into a desired
20 form. The versatility of the synthetic tissue and the complex of the present invention should be noted.

 The synthetic tissue and the complex of the present invention have biological integration with recipient
25 tissues, such as surrounding tissues, cells, and the like. Therefore, the post-operational acceptance is satisfactory, and cells are reliably supplied to a local site, for example. An effect of the present invention is that the satisfactory biological integration capability allows the formation of
30 a tissue complex with another synthetic tissue or the like, resulting in a complicated therapy.

 Another effect of the present invention is that

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differentiation can be induced after the synthetic tissue or the complex is provided. Alternatively, differentiation is induced before providing a synthetic tissue and/or a complex, and thereafter, the synthetic tissue and/or the complex are formed.

Another effect of the present invention is that the cell implantation of the present invention provides a satisfactory replacement ability and a comprehensive supply of cells for covering an implanted site, compared to conventional cell-only implantation and sheet implantation.

The present invention provides an implantable synthetic tissue. The above-described features and effects of the present invention become it possible to treat a site which cannot be considered as an implantation site for conventional synthetic products. The present invention makes it possible to provide a synthetic tissue or a three-dimensional structure using not only a heart muscle but also cells derived from other parts. The synthetic tissue of the present invention has biological integration and actually works in implantation therapies. The synthetic tissue is first provided by the present invention, but is not provided by conventional techniques.

In addition, the present invention provides medical treatment which provides a therapeutic effect by filling, replacing, and/or covering an affected portion.

In addition, when the synthetic tissue of the present invention is used in combination with another synthetic tissue (e.g., an artificial bone made of hydroxyapatite, a microfibrinous collagen medical device, etc.), the synthetic

tissue of the present invention is biologically integrated with the other synthetic tissue, so that the acceptance of the synthetic tissue can be improved to an extent which is not conventionally expected.

5

An extracellular matrix or a cell adhesion molecule, such as fibronectin, vitronectin, or the like, is distributed throughout the synthetic tissue of the present invention. In the cell sheet engineering, a cell adhesion molecule is localized on a surface of culture cells which is attached to a culture dish. In the sheet of the cell sheet engineering, cells are major components of the sheet. The sheet is nearly a mass of cells, on the bottom surface of which an adhesion molecule (glue) is added. The synthetic tissue of the present invention is a real "tissue" such that an extracellular matrix wraps cells. Thus, the present invention is significantly distinguished from conventional techniques.

A cell implanting method without a scaffold has been reported by Kushida A., Yamato M., Konno C., Kikuchi A., Sakurai Y., Okano T., J. Biomed. Mater. Res., 45:355-362, 1999, in which a cell sheet is produced using a temperature sensitive culture dish. Such a cell sheet engineering technique is internationally appraised due to its originality. However, a single sheet obtained by this technique is fragile. In order to obtain the strength that can withstand surgical manipulation, such as implantation, a plurality of sheets need to be assembled, for example. Such a problem is solved by the present invention.

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A cell/matrix complex developed by the present invention does not require a temperature sensitive culture dish unlike the cell sheet technique. The cell/matrix

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complex is easily formed into a contractile three-dimensional tissue. There is no technique in the world other than the present invention, which can produce a contractile three-dimensional complex having 10 or more layers without using so-called feeder cells, such as rodent stroma cells, after approximately three weeks. By adjusting conditions for matrix production of the synovial cell, it is possible to produce a complex having a strength which allows surgical manipulation, such as holding or transferring the complex, without a special instrument. Therefore, the present invention is an original, epoch-making technique in the world for reliably and safely performing cell implantation.

In a preferable embodiment, the synthetic tissue of the present invention has a biological integration capability to the surroundings. As used herein the term "surroundings" typically means surroundings to be implanted, and examples thereof include tissues, cells and the like. The biological integration capability with surrounding tissues, cells, and the like can be confirmed by, for example, photomicrograph, physical test, staining of a biological marker, or the like. Conventional synthetic tissues have a low affinity for adjacent tissues in which they are implanted. It was not even assumed that conventional synthetic tissues have the biological integration capability. Conventional synthetic tissues depend on a regeneration capability of an organism, and serves as a temporary solution until autologous cells gather and regenerate. These conventional synthetic tissues are not intended to for a permanent use. Therefore, the synthetic tissue of the present invention should be contemplated as an implantation treatment in the true sense. The biological integration capability referred to by in the present invention preferably includes an adhesion capability

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to surrounding cells. Such an adhesion capability can be measured by an *in vitro* culturing assay (see Figure 23) with a tissue section (e.g., a cartilage section).

As used herein, the term "disease" to be treated by the present invention refers to any disease accompanying degeneration, necrosis, injury or the like, and examples thereof including, osteoarthritis, osteochondral injury, intractable fracture, osteonecrosis, cartilage injury, meniscus injury, ligament injury, tendon injury, cartilage degeneration, meniscus degeneration, intervertebral disk denaturation, ligament degeneration, or tendon degeneration, or any heart diseases having an injured tissue. Examples of such heart diseases include heart failure, intractable heart failure, myocardial infarct, cardiomyopathy, dilated cardiomyopathy, hypertrophic cardiomyopathy, dilated phase hypertrophic cardiomyopathy, and the like. The combined therapy of the present invention may be applied to a regeneration of an injury in an organ other than a heart, as long as regeneration of a tissue injury is the goal. In a specific embodiment, a disease to be treated by the method of the present invention is intractable heart failure.

As used herein, the term "prophylaxis" or "prevention" in relation to a certain disease or disorder refers to a treatment which keeps such a condition from happening before the condition is caused, or causes the condition to occur at a reduced level or to be delayed.

As used herein, the term "therapy" in relation to a certain disease or disorder means that when such a condition occurs, such a disease or disorder is prevented from deteriorating, preferably is retained as it is, more preferably is diminished, and even more preferably

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extinguished. As used herein, the term "radical therapy" refers to a therapy which eradicates the root or cause of a pathological process. Therefore, when a radical therapy is made for a disease, there in principle is no recurrence of the disease.

As used herein, the term "prognosis" is also referred to as "prognostic treatment". The term "prognosis" in relation to a certain disease or disorder refers to a diagnosis or treatment of such a condition after a therapy.

In a preferable embodiment, the synthetic tissue or complex of the present invention has a three-dimensional, biological integration. As described in other portions of the specification, examples of biological integration include, but are not limited to, physical integration or connection via extracellular matrices, electrical integration, and the like. Particularly, in a preferable embodiment including the cells, it is important that extracellular matrix in a tissue is biologically organized. Such a synthetic tissue which is biologically organized has not been provided. Thus, the synthetic tissue of this embodiment according to the present invention is new also in view of the structure. Further, the preferable embodiment having a biological integration capability with the surroundings provides a synthetic tissue which has not exist conventionally on the point that the synthetic tissue can form a part of an organism after implantation. The present invention can provide an synthetic tissue which does not include any cell, even a cell which has been frozen once and died. The tissue is still unique on the point that it has an affinity with the surrounding even in such a case.

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In one embodiment, the synthetic tissue of the present invention is different from conventional synthetic tissues in that the former comprises a cell. Particularly, a high density that the density of $5 \times 10^6/\text{cm}^2$ at maximum can be included is important. The present invention is important on the point that it is suitable for implanting cells rather than implanting the tissue.

Preferably, a synthetic tissue of the present invention substantially comprise cells or a material derived from the cells. Since the synthetic tissue is composed substantially of only cells and a cell-derived material (e.g., extracellular matrix, etc.), the synthetic tissue can have an increased level of biocompatibility and affinity. As used herein, the terms "substantially comprise ...", "substantially made of ...", and "substantially contain ..." mean that cells and substances derived from the cells are included, and also any other substance may be included as long as it does not cause any harmful effect (herein, mainly, bad effect on implantation), and should be understood as such herein. Such substances which do not cause any harmful effect are known to those skilled in the art or can be confirmed by conducting an easy test. Typically, such substances are, but not limited to, any additives permitted by the Health, Labor and Welfare Ministry, Food and Drug Administration (FDA) or the like, ingredients involved in cell culture, and the like. The cell-derived material representatively includes extracellular matrices. Particularly, the synthetic tissue or complex of the present invention preferably comprises a cell and an extracellular matrix at an appropriate ratio thereof. Such an appropriate ratio of a cell and an extracellular matrix is from about 1:3 to about

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20:1. The strength of the tissue is adjusted by the ratio between a cell and an extracellular matrix. The ratio between a cell and an extracellular matrix is adjusted for use in accordance with application of cell implantation and physical environment at the implantation site. Preferable ratio varies depending on the treatment to be aimed. Such a variation is apparent to those skilled in the art and can be estimated by investigating the ratio of a cell in an organ which is a target and an extracellular matrix.

Preferably, a synthetic tissue substantially comprising cells and an extracellular matrix derived from the cells has not been known. Therefore, the present invention provides a totally new synthetic tissue.

Preferably, an extracellular matrix which forms the present invention includes, collagen I, collagen III, vitronectin, fibronectin, and the like. It is preferable that a variety of extracellular matrix includes all the listed ingredients, and that they are integrated and mixed. Alternatively, it is preferable that extracellular matrix is dispersed across the entire body. Such a distribution has a significant effect on the point that compatibility and affinity with the environment can be improved when implanted. The present invention is known to be characterized in that adhesion to intercellular matrix which promotes cell adhesion to a matrix, cell extension, and cell chemotaxis is also promoted by including collagen (Types I, III), vitronectin, fibronectin, and the like. However, a synthetic tissue which includes collagen (Types I, III), vitronectin, fibronectin, and the like has not been provided. It is not intended to be constrained by the theory, but, collagen (Types I, III), vitronectin, fibronectin, and the

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like are contemplated to have a function in exercising the biological integration capability with the surrounding. Therefore, in the preferable embodiment, it is advantageous that vitronectin are positioned to be dispersed on a surface
5 of the synthetic tissue or complex of the present invention. It is considered that adhesion, affinity, and stability after implantation are significantly different.

It is preferable that the fibronectin is also
10 positioned in the synthetic tissue or complex of the present invention. It is known that fibronectin has a function in cell adhesion, control of a shape of a cell, and adjustment in cell migration. A synthetic tissue in which fibronectin is expressed has not been provided. It is not intended to
15 be constrained by the theory, fibronectin is also contemplated to have a function in exercising the biological integration capability with the surrounding. Therefore, in the preferable embodiment, it is advantageous that fibronectin are also positioned to be dispersed on a surface
20 of the synthetic tissue or complex of the present invention. It is considered that adhesion, affinity, and stability after implantation are significantly different.

In the preferred embodiment, it is understood that
25 to position extracellular matrix used in the present invention on the synthetic tissue or complex can be readily achieved by the synthetic tissue production method of the present invention. It is also understood that the production method is not limited to this.

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In more preferable embodiment, it is advantageous to position the extracellular matrix used in the present invention to be dispersed. Positioning extracellular

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matrix into such a dispersed state was impossible in conventional synthetic tissues. It is understood the present invention is the first to provide such a tissue.

5 In the preferred embodiment, regarding extracellular matrix positioned to be dispersed on the synthetic tissue or complex, when distribution densities in any two section of 1cm^2 are compared, the ratio is preferably within the range of about 1:3 to 3:1. Measurement of
10 distribution densities can be performed by any method known in the field of the art, for example, immune staining or the like.

 In the preferred embodiment, regarding
15 extracellular matrix used in the present invention, when distribution densities in any two section of 1cm^2 are compared, the ratio is preferably within the range of about 1:2 to 2:1, and further preferably, about 1.5:1 to 1.5:1. It is advantageous that extracellular matrix is uniformly
20 dispersed. Preferably, extracellular matrix is dispersed substantially uniform, but it is not limited to this.

 In one embodiment, extracellular matrix positioned in the present invention may include collagen I, collagen
25 III, vitronectin, fibronectin or the like.

 In an alternative embodiment, the synthetic tissue or complex of the present invention may employ heterologous cells, allogenic cells, isogenic cells or autologous cells.
30 In the present invention, it is found that even allogenic cells, particularly, mesenchymal cells are used, no adverse reactions, such as immune rejection reactions, is generated. Thus, the present invention ends to the development of the

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treatment of *ex vivo*, and also a therapy which produces a synthetic tissue using cells of others and utilize the tissue without using an immuno rejection suppressor or the like.

5 In one preferred embodiment, the cells included in the synthetic tissue or complex of the present invention may be stem cells, differentiation cells, or they may include both. In the preferred embodiment, the cells included the three directional sturcture are mesenchymal cells. It is
10 not intended to restrained to the theory, the mesenchymal cells are preferably used because the mesenchymal cells are highly compatible with various organs such as heart, and may have capability to differentiate into various organs such as a heart.

15 Such mesenchymal cells may be mesenchymal stem cells, or may be mesenchymal differentiation cells.

 Examples of the mesenchymal cells used in the present
20 invention include, but not limited to, bone marrow cells, adipocyte, synovial cell, myoblast, skeletal muscle cells, and the like. Examples of mesenchymal cells as used herein include stem cells derived from an adipose tissue, stem cells derived from a bone marrow, and the like.

25 In the preferred embodiment, it is advantageous that the cells used in the present invention are cells derived from the subject to which the synthetic tissue or complex is applied. In such a case, cells as used herein also referred
30 to as autologous cells. By using autologous cells, immune rejection reactions can be prevented or reduced.

 Alternatively, in another embodiment, the cells as

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used herein may not be cells derived from a subject to which the synthetic tissue or complex is applied. In such a case, it is preferable that measures are taken to prevent immune rejection reactions.

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The synthetic tissue or complex of the present invention may be provided as a drug. Alternatively, the synthetic tissue or complex may be prepared by a physician for therapy, or, a physician may first prepare the cells, and then the third party may culture the cells and prepare as a third-dimension structure for use in a surgery. In such a case, culturing cells is not necessarily performed by a physician, but can be performed by those skilled in the art of cell culture. Those skilled in the art can determine culturing conditions in accordance with a variety of the cells and an implantation site to be targeted after reading the disclosure herein.

In another embodiment, the synthetic tissue or complex of the present invention is preferably isolated. In this case, the term "isolate" means that the synthetic tissue is detached from a scaffold, a support, and a culture medium used in culture. If a synthetic tissue of the present invention is substantially free of materials, such as a scaffold and the like, it is possible to suppress adverse reactions after implantation, such as immune rejection reactions, inflammation reactions, and the like.

The base area of the synthetic tissue according to the present invention may be, for example, 1 cm^2 to 20 cm^2 . However, the area is not limited to this range and may be smaller than 1 cm^2 , or greater than 20 cm^2 . It is understood

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that the essential feature of the present invention is that a tissue of any size (area, volume) can be produced, and it is not limited in the size.

5 In a preferable embodiment, the synthetic tissue of the present invention is thick. The term "thick" in relation to a synthetic tissue typically means that the synthetic tissue has a thickness which provides a strength sufficient to cover a site to which the synthetic tissue is implanted.
10 Such a thickness is, for example, at least about 50 μm , more preferably at least about 100 μm , at least about 200 μm , at least about 300 μm , even more preferably at least about 400 μm , still more preferably at least about 500 μm , and still even more preferably about 1 mm. It is recognized that, in some
15 cases, a tissue having a thickness of 3 mm or greater and a tissue having a thickness of 5 mm or greater can be produced. Alternatively, such a thickness may be, 1 mm or less. It is understood that an essential feature of the present invention is that a tissue or a complex having any thickness
20 can be produced, and the tissue or complex is not limited in the size.

The present invention provides a scaffold-free synthetic tissue or complex. By providing such a
25 scaffold-free synthetic tissue, a therapeutic method and a therapeutic agent for providing an excellent condition after implantation can be obtained.

The scaffold-free synthetic tissue of the present
30 invention solves a long outstanding problem with biological formulations, which is attributed to contamination of the scaffold itself. Despite the absence of a scaffold, the therapeutic effect is comparable with or more satisfactory

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than conventional techniques.

In addition, when a scaffold is used, the alignment of implanted cells in the scaffold, the cell-to-cell adhesion, the in vivo alteration of the scaffold itself (eliciting inflammation), the acceptance of the scaffold to recipient tissue, and the like become problematic. These problems can be solved by the present invention.

The synthetic tissue and the complex of the present invention are also self-organized, and have biological integration inside thereof. Also in this point, the present invention is distinguished from conventional cell therapies.

The synthetic tissue and the complex of the present invention are easy to form a three-dimensional structure, and is thus easy to be designed into a desired form. The versatility of the synthetic tissue and the complex of the present invention should be noted.

The synthetic tissue and the complex of the present invention have biological integration with recipient tissues, such as surrounding tissues, cells, and the like. Therefore, the post-operational acceptance is satisfactory, and cells are reliably supplied to a local site, for example. An effect of the present invention is that the satisfactory biological integration capability allows the formation of a tissue complex with another synthetic tissue or the like, resulting in a more complex therapy.

Another effect of the present invention is that differentiation can be induced after the synthetic tissue or the complex is provided. Alternatively, differentiation

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is induced before providing a synthetic tissue and/or a complex, and thereafter, the synthetic tissue and/or the complex are formed.

5 Another effect of the present invention is that the cell implantation of the present invention provides a satisfactory replacement and a comprehensive supply of cells for covering an implanted site, compared to conventional cell-only implantation and sheet implantation.

10 The present invention provides an implantable synthetic tissue having biological integration capability. The above-described features and effects of the present invention become it possible to treat a site which cannot
15 be considered as an implantation site for conventional synthetic products. The present invention makes it possible to provide a synthetic tissue or a three-dimensional structure. The synthetic tissue of the present invention has biological integration and actually works in implantation
20 therapies. The synthetic tissue is first provided by the present invention, but is not provided by conventional techniques.

25 In addition, the present invention provides medical treatment which provides a therapeutic effect by filling, replacing, and/or covering an affected portion.

30 In addition, when the synthetic tissue of the present invention is used in combination with another synthetic tissue (e.g., an artificial bone made of hydroxyapatite, a microfibrous collagen medical device, etc.), the synthetic tissue of the present invention is biologically integrated with the other synthetic tissue, so that the acceptance of

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the synthetic tissue can be improved to an extent which is not conventionally expected.

5 An extracellular matrix or a cell adhesion molecule, such as fibronectin, vitronectin, or the like, is distributed throughout the synthetic tissue of the present invention. In cell sheet engineering, a cell adhesion molecule is localized on a surface of culture cells which is attached to a culture dish. In the sheet of the cell sheet engineering, 10 the cells are major components of the sheet. The sheet is nearly a mass of cells, on the bottom surface of which an adhesion molecule (glue) is added. On the other hand, the synthetic tissue of the present invention is a real "tissue" such that an extracellular matrix covers cells. Thus, the 15 present invention is significantly distinguished from conventional techniques.

A cell implanting method without a scaffold has been reported by Kushida A., Yamato M., Konno C., Kikuchi A., 20 Sakurai Y., Okano T., J. Biomed. Mater. Res., 45:355-362, 1999, in which a cell sheet is produced using a temperature sensitive culture dish. Such a cell sheet engineering technique is internationally appraised due to its originality. However, a single sheet obtained by this technique is fragile. 25 In order to obtain the strength that can withstand surgical manipulation, such as implantation, a plurality of sheets need to be assembled, for example. Such a problem is solved by the present invention.

30 A cell/matrix complex developed by the present invention does not require a temperature sensitive culture dish unlike the cell sheet technique. The cell/matrix complex is easy to form into a contractile three-dimensional

tissue. There is no technique in the world other than the present invention, which can produce a contractile three-dimensional complex having 10 or more layers without using so-called feeder cells, such as rodent stroma cells, at about three weeks. By adjusting conditions for matrix production of the cell, it is possible to produce a complex having a strength which allows surgical manipulation, such as holding or transferring the complex, without a special instrument. Therefore, the present invention is an original, epoch-making technique in the world for reliably and safely performing cell implantation.

In another embodiment, the synthetic tissue or complex of the present invention is flexible. Due to the flexibility, the synthetic tissue is particularly suitable for reinforcement of motile organs. Examples of motile organs include, but are not limited to, hearts, blood vessels, muscles, and the like.

In another embodiment, the synthetic tissue or complex of the present invention has dilation/contraction ability. Due to the dilation/contraction ability, the synthetic tissue is suitable for organs which expand and contract, including, for example, hearts, muscles, and the like. The dilation/contraction ability cannot be achieved by cell sheet or the like prepared by conventional methods. Preferably, a synthetic tissue of the present invention has a sufficient strength to withstand the pulsation motion of a heart. The strength sufficient to withstand pulsation motion is, but is not limited to, at least about 50% of the strength of naturally-occurring myocardium, preferably at least about 75%, and more preferably at least about 100%.

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In a preferable embodiment, the synthetic tissue or complex of the present invention has biological integration in all three dimensions. There are some synthetic tissues prepared by conventional methods, which have biological integration in two dimensions to some degree. However, no tissue having biological integration in all three dimensions can be prepared by conventional methods. Therefore, since the synthetic tissue of the present invention has biological integration in all three dimensions, the synthetic tissue is substantially implantable in any application.

Examples of biological integration which is an indicator of a synthetic tissue or complex of the present invention, include, but are not limited to, interconnection of extracellular matrices, electrical integration, the presence of intracellular signal transduction, and the like. The interaction of extracellular matrices can be observed with a microscope by staining intracellular adhesion as appropriate. Electrical integration can be observed by measuring electric potential.

In a preferable embodiment, the synthetic tissue of the present invention has a sufficient tissue strength for clinical applications. The sufficient tissue strength for clinical applications varies depending on a site to which the synthetic tissue is applied. Such a strength can be determined by those skilled in the art with reference to the disclosure of the specification and techniques well known in the art. The tensile strength of the synthetic tissue of the present invention may be low. The tensile strength becomes higher when the matrix concentration is increased, and becomes lower when the cell ratio is increased. The present invention is characterized in that the strength can

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be adjusted as necessary. The present invention is also characterized in that the strength can approximate to be high or low relative to that of a tissue to be implanted. Therefore, it is recognized that the goal can be set to comply
5 with any site.

In another embodiment, it is preferable that a strength of the synthetic tissue or complex is sufficient for having a self-supporting ability. Conventional
10 synthetic tissues do not have a self-supporting ability after production. Therefore, when conventional synthetic tissues are transferred, at least a part of them are injured. However, when the technique of the present invention is used, the synthetic tissue having the self-supporting ability is
15 provided. This means that the present invention provides the synthetic tissue which cannot be provided by conventional techniques. Preferable self-supporting ability is such that, when a tissue is picked up with a tweezers having tips of 0.5 to 3 mm (preferably, tips of 1 to 2 mm, and more
20 preferably, tips of 1 mm), the tissue is not substantially destroyed. Herein, whether the tissue is not substantially destroyed can be confirmed with eyes, but can be confirmed by performing, for example, a water leakage test after the tissue is picked up in the above-described conditions and
25 confirming that water does not leak. Alternatively, the self-supporting ability as described above can also be confirmed by not being destroyed when picked up by fingers, instead of tweezers.

30 In a particular embodiment of the present invention, the above-described clinical application is intended to a bone, a joint, a cartilage, a meniscus, a tendon, a ligament, a kidney, a liver, a synovial membrane, a heart, and the

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like. The origin of cells contained in the synthetic tissue of the present invention is not affected by clinical applications.

5 Also, when a synthetic tissue of the present invention is applied to a cartilage, the attachment ability of the synthetic tissue can be tested by determining whether or not the synthetic tissue remains attached without an additional fixation procedure when the synthetic tissue is
10 implanted into an injured portion of the intra-articular tissue (e.g., 2, 3 minutes after).

 In another aspect, the present invention provides a cell culture composition for producing synthetic tissue
15 from a cell. The cell culture composition contains an ingredient (e.g., commercially available medium, etc.) for maintaining or growing the cell, and an ECM synthesis promoting agent. The ECM synthesis promoting agent has been described in detail in the above description of the synthetic
20 tissue production method. Therefore, the ECM synthesis promoting agent includes ascorbic acid or a derivative thereof (e.g., TGF- β 1, TGF- β 3, ascorbic acid 1-phosphate or a salt thereof, ascorbic acid 2-phosphate or a salt thereof, L-ascorbic acid or a salt thereof, etc.). The culture
25 composition of the present invention contains ascorbic acid 2-phosphate or a salt thereof at a concentration of at least 0.1 mM. Alternatively, in the case of a condensed culture composition, the condensed culture composition contains ascorbic acid 2-phosphate or a salt thereof at a concentration
30 which becomes at least 0.1 mM after preparation. Ascorbic acid 2-phosphate or a salt thereof contained in the culture composition of the present invention is present at a concentration of at least 0.1 mM. When the culture

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composition of the present invention is condensed, ascorbic acid 2-phosphate or a salt thereof contained therein is present at a concentration of at least 0.1 mM after formulation. It seems that 0.1 mM or more ascorbic acids have substantially a constant effect. Thus, 0.1 mM can be said to be sufficient. For TGF- β 1 and TGF- β 3, 1 ng/ml or more, representatively 10 ng/ml, may be sufficient.

Alternatively, the present invention may provide a composition for producing a synthetic tissue, comprising such an ECM synthesis promoting agent.

In another embodiment of the present invention, an ECM synthesis promoting agent used in the synthetic tissue production method of the present invention includes ascorbic acid 2-phosphate (Hata R., Sano H., J. Cell Physiol., 1989, 138(1):8-16). In the present invention, by adding an at least predetermined amount of ascorbic acid 2-phosphate, the production of an extracellular matrix is promoted. As a result, the resultant synthetic tissue or complex is made rigid, and therefore, becomes easy to be detached. Thereafter, the tissue undergoes self-contraction in response to a stimulus of detachment. Hata et al. does not disclose that the culture in medium supplemented with ascorbic acid causes the tissue to be rigid and thus confers to the tissue a property of being easily detached. Though not wishing to be bound by any theory, a significant difference between the present invention and Hata et al. is present in cell density. Also, Hata et al. does not suggest the effect of facilitating detachment of cells from a container for culture. The present invention is the first to find the effect of tissue contraction on development of three-dimensional synthetic tissue from monolayer cultured

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cells. The synthetic tissue of the present invention can be absolutely distinguished from conventional synthetic tissues, since the synthetic tissue of the present invention is produced via the procedures of tissue detachment and subsequent tissue contraction.

In a preferable embodiment, ascorbic acid 2-phosphate used in the present invention is typically present at a concentration of at least 0.01 mM, preferably at least 0.05 mM, more preferably at least 0.1 mM, even more preferably at least 0.2 mM, and still more preferably at least 0.5 mM, and still even more preferably 1.0 mM.

In one embodiment of the present invention, the cell density is, but is not particularly limited to, 5×10^3 to 5×10^6 cells per 1 cm^2 . These conditions may be, for example, applied to myoblast. In this case, preferably, the ECM synthesis promoting agent may be ascorbic acids and may be provided at a concentration of at least 0.1 mM. This is because a thick synthetic tissue can be produced. In this case, if the concentration is increased, a synthetic tissue having a dense extracellular matrix is produced. If the concentration is low, the amount of an extracellular matrix is decreased but the self-supporting ability is maintained.

(Synthetic tissue for replacement and coverage)

In another aspect, the present invention provides a synthetic tissue or complex for reinforcement of a portion of an animal organism. The synthetic tissue or complex capable of such reinforcement is a technique achieved only after the synthetic tissue production method of the present invention is provided. Since the synthetic tissue or complex of the present invention has self-supporting ability, it

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can be used in applications which are not conventionally provided (e.g., filling (replacement) reinforcement, whole reinforcement, no-leakage reinforcement, coverage, etc.). The present invention has a significant effect such that the filling and replacement reinforcement (i.e., cell supply) was significantly improved. The present invention also allows differentiation induction, which enlarges the range of application of the present invention.

In a specific embodiment of the present invention, the above-described reinforcement may be achieved by disposing a synthetic tissue of the present invention to cover the above-described portion. It is not possible to use a synthetic tissue provided by conventional methods to perform treatment by covering the above-described portion (i.e., replacement and/or coverage application). Thus, the synthetic tissue of the present invention can provide applications which cannot be achieved by conventional techniques.

Therefore, in the above-described specific embodiment, the synthetic tissue or complex of the present invention is resistant to dilation/contraction of the above-described portion.

In a preferable embodiment, the synthetic tissue or complex of the present invention advantageously has biological integration.

In another preferable embodiment, the biological integration includes at least one of interconnection of extracellular matrices, electrical integration, and intracellular signal transduction.

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5 In another preferable embodiment, the synthetic tissue or complex for reinforcement of the present invention is formed by culturing a cell in the presence of an ECM synthesis promoting agent.

10 In another embodiment, the synthetic tissue or complex for reinforcement of the present invention comprises a cell (autologous cell) derived from an animal to be treated (e.g., a human). More preferably, a synthetic tissue for reinforcement of the present invention comprises only a cell(s) (autologous cell) derived from an animal to be treated (e.g., a human) as a cell source.

15 Applications for the therapy utilizing the present invention include, for example: cartilage full thickness injury, cartilage partial injury; osteochondral injury; osteonecrosis; osteoarthritis; meniscus injury; ligament injury (chronic injury, degenerative tear, biological augmentation for reconstruction surgery, etc.); rotator cuff (particularly, chronic injury, degenerative tear, etc.); delayed union; nonunion; skeletal muscle repair/regeneration; cardiac muscle repair; (augmenting the repair of necrotic tissue by ischemic-heart disease) or the like.

(Therapy using replacement and coverage)

30 In another aspect, the present invention provides a method for reinforcement of a portion of an animal organism. The method comprises the steps of: A) disposing a synthetic tissue or complex to replace or cover the portion; and B) holding the synthetic tissue or complex for a time sufficient to connect to the portion. Herein, to position

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a portion for replacement typically means to perform debridement or curettage of an affected portion as necessary, to position the synthetic tissue or complex of the present invention on the lesion, and to allow it to stand so as to promote replacement. An objective of such replacement is to fill cells. Techniques known in the art can be combined and used. The step of disposing the synthetic tissue to cover the portion can be carried out using a technique well known in the art. The sufficient time varies depending on a combination of the portion and the synthetic tissue, and can be easily determined as appropriate by those skilled in the art depending on the combination. Examples of such a time include, but are not limited to, 1 week, 2 weeks, 1 month, 2 months, 3 months, 6 months, 1 year, and the like. In the present invention, a synthetic tissue preferably comprises substantially only cell(s) and material(s) derived from the cell. Therefore, there is no particular material which needs to be extracted after operation. The lower limit of the sufficient time is not particularly important. In this case, it can be said that the longer the time, the more preferable the synthetic tissue. If the time is sufficiently extremely long, it can be said that reinforcement is substantially completed. Therefore, the time is not particularly limited. The synthetic tissue of the present invention is also characterized in that it is easily handled, is not destroyed during an actual treatment, and facilitates a surgery due to its self-supporting ability.

In another embodiment, in a reinforcement method of the present invention, the above-described portion preferably includes bag-shaped organs (e.g., hearts, livers, kidneys, etc.). In order to reinforce such a bag-shaped tissue, it is necessary to replace or cover the organ. A

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synthetic tissue resistant to applications for replacement or covering is first provided by the present invention. Therefore, the reinforcement method of the present invention is advantageous over conventional techniques.

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Alternatively, the above-described portion may include a bone or cartilage. Examples of such portions include, but not limited to, meniscus, ligament, tendon, and the like. By the method of the present invention a disease, injury, or condition of a heart, bone, cartilage, 10 ligament, tendon, or meniscus may be treated, prevented or reinforced.

Particularly, in the reinforcement method of the present invention, a synthetic tissue or complex of the present invention is resistant to dilation/contraction of the above-described portion. Examples of such dilation/contraction include, but are not limited to, the pulsation motion of a heart, the contraction of a muscle, 15 and the like.

In another preferable embodiment, in the reinforcement method of the present invention, a synthetic tissue or complex of the present invention has biological integration (e.g., interconnection of extracellular 25 matrices, electrical integration, intracellular signal transduction, etc.). The biological integration is preferably provided in all three dimensions.

30 In another preferable embodiment, the reinforcement method of the present invention further comprises culturing a cell in the presence of an ECM synthesis promoting agent to form a synthetic tissue or complex of the present invention.

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An implantation/regeneration technique using the method which comprises the step of culturing a cell in the presence of an ECM synthesis promoting agent cannot be provided by conventional techniques. The method provides a therapy for
5 diseases (e.g., , cartilage injury, intractable bone fracture, etc.), which cannot be achieved by conventional therapies.

In a preferable embodiment, in the reinforcement method of the present invention, the cell used in the synthetic
10 tissue or complex of the present invention is derived from an animal to which the synthetic tissue is to be implanted (i.e., an autologous cell). By using an autologous cell, adverse side effects, such as immune rejection reactions or the like, can be avoided.

15 In another preferable embodiment, the portion is a heart.

Applications for the therapy utilizing the present
20 invention include, for example: cartilage full thickness injury, cartilage partial injury; osteochondral injury; osteonecrosis; osteoarthritis; meniscus injury; ligament injury (chronic injury, degenerative tear, biological augmentation for reconstruction surgery, etc.); rotator cuff
25 (particularly, chronic injury, degenerative tear, etc.); delayed union; nonunion; skeletal muscle repair/regeneration; cardiac muscle repair; (augmenting the repair of necrotic tissue by ischemic-heart disease) or the like.

30 For some organs, it is said that it is difficult to radically treat a specific disease, disorder, or condition thereof (e.g., refractory heart diseases). However, the

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present invention provides the above-described effect, thereby making possible a treatment which cannot be achieved by conventional techniques. It has been clarified that the present invention can be applied to radical therapy.

5 Therefore, the present invention has usefulness which cannot be achieved by conventional medicaments.

Thus, the present invention provides a method for treating a portion of an organism of an animal, comprising:

10 A) positioning the synthetic tissue or complex so as to cover the portion; and B) retaining the synthetic tissue for a time period which is sufficient for the condition of the portion of the organism to be improved. Such an improvement in the condition can be determined in accordance with the function of the portion to be treated.

15 For example, when a heart should be treated, an improvement in the condition can be determined by checking a cardiac function (heartbeat, bloodstream, or the like). If a bone should be treated, an improvement in the condition can be determined by observing osteogenesis by using roentgen, CT scan, or the like. In the case of a bone, an improvement in the condition can be determined by measuring its strength or by evaluating bone marrow and/or a bone substance by using MRI.

20 If a cartilage or meniscus should be treated, a surface of a joint can be observed by an arthroscopy. Further, it is possible to determine an improvement in the condition by performing a biomechanical inspection under arthroscopy. It is also possible to determine an improvement in the condition by confirming a repairing condition by using MRI.

25 Regarding ligament, it is possible to determine by confirming whether there is laxity by a joint stability inspection. Further, an improvement of the condition can be determined by confirming a continuousness of a tissue by an MRI. In

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the case of any tissue, it is possible to determine whether the condition is improved by performing a biopsy of the tissue and making a histological evaluation.

5 In a preferred embodiment the treatment treats, prevents, prognosis, or enhances a disease, injury, or condition of a heart, bone, cartilage, ligament, tendon, or meniscus. Preferably, the synthetic tissue or the complex has a self-supporting ability. For such a synthetic tissue,
10 those skilled in the art can use a synthetic tissue of any form described above herein, and a variant thereof.

(Combined therapy)

15 In another aspect, the present invention provides a regeneration therapy which uses a cytokine, such as BMP (e.g., BMP-2, BMP-4, BMP-7, etc.), TGF- β 1, TGF- β 3, HGF, FGF, IGF, or the like, in combination with a synthetic tissue.

20 Some cytokines used in the present invention are already commercially available (e.g., BMP (Yamanouchi Pharmaceutical), bFGF2 (Kaken Pharmaceutical), TGF- β 1 (for research only, HGF-101 from Toyo Boseki, etc.)). However, these cytokines can be prepared by various methods and can be used in the present invention if they are purified to
25 an extent which allows them to be used as a medicament. A certain cytokine can be obtained as follows: primary cultured cells or an established cell line capable of producing the cytokine is cultured; and the cytokine is separated from the culture supernatant or the like, followed by
30 purification. Alternatively, a gene encoding the cytokine is incorporated into an appropriate vector by a genetic engineering technique; the vector is inserted into an appropriate host to transform the host; a recombinant

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cytokine of interest can be obtained from the supernatant of the transformed host culture (e.g., Nature, 342, 440(1989); Japanese Laid-Open Publication No. 5-111383; Biochem-Biophys. Res. Commun., 163, 957 (1989), etc.). The
5 above-described host cell is not particularly limited and can be various host cells conventionally used in genetic engineering techniques, including, for example, *Escherichia coli*, yeast, animal cells, and the like. The thus-obtained cytokine may have one or more amino acid substitutions,
10 deletions and/or additions in the amino acid sequence as long as it has substantially the same action as that of the naturally-occurring cytokine. Examples of a method for introducing the cytokine into patients in the present invention include, but are not limited to, a Sendai virus
15 (SVJ) liposome method with high safety and efficiency (Molecular Medicine, 30, 1440-1448(1993); Jikken Igaku (Experimental Medicine), 12, 1822-1826 (1994)), an electrical gene introduction method, a shotgun gene introduction method, a ultrasonic gene introduction method,
20 and the like. In another preferable embodiment, the above-described cytokines can be administered in the form of proteins.

(Production method of synthetic tissue having
25 desired thickness)

Another aspect of the present invention provides a method for producing a synthetic tissue or complex having a desired thickness. This method comprises: A) providing cells; B) positioning the cells in a container having the
30 base area sufficient for accommodating the synthetic tissue or complex having the desired size, which contains an ECM synthesis promoting agent (e.g., ascorbic acids, TGF- β 1, TGF- β 3, etc.); C) culturing the cells in the container with

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a cell culture medium including the ECM synthesis promoting agent for a time sufficient for forming the synthetic tissue or complex having the desired size to convert the cells into a synthetic tissue; and D) adjusting the thickness of the synthetic tissue to obtain a desired thickness by a physical stimulation or a chemical stimulation. Herein, the steps of providing the cells, positioning the cells, stimulating and converting into the tissue or complex are described with respect to the production method for the synthetic tissue or complex of the present invention in detail, and it is understood that any embodiment can be employed.

Next, examples of the physical or chemical stimulation to be used may include, but not limited to, use of pipetting, use of actin interacting substance. Pipetting may be preferable because operation is easy and no harmful substance is produced. Alternatively, examples of the chemical stimulation to be used may include actin depolymerizing factors and actin polymerizing factor. Examples of such an actin depolymerizing factor may include ADF(actin depolymerizing factor), destrin, depactin, actophorin, cytochalasin, NGF(nerve growth factor) and the like. Examples of the actin polymerizing factor include LPA(lysophosphatidic acid), insulin, PDGFa, PDGFb, chemokine, and TGFb. The polymerization or depolymerization of actin can be observed by checking the activity to actin. It is possible to test any substance whether it has such an activity. It is understood that a substance which is tested as such and identified can be used for achieving the desired thickness in production of the synthetic tissue of the present invention. For example, in the present invention, the adjustment of the desired thickness can be achieved by adjusting the ratio between

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the actin depolymerizing factor and actin polymerizing factor.

(Composite tissue)

5 Another aspect of the present invention also provides a tissue complex including an implantable synthetic tissue and another synthetic tissue. Herein, another tissue may either be a synthetic tissue included within the scope of the present invention, or a synthetic tissue out of the scope
10 (i.e., conventional tissues). Conventional tissues (e.g., an artificial bone, microfibrous collagen medical device, etc.,) do not have a biological integrating ability or have a biological integrating ability which cannot stand the practical use. Thus, it was almost impossible to form such
15 a tissue complex. It is understood that, according to the present invention, a cartilage can be combined to a bone for treatment. For the case of a cavity in a bone or the like, particularly, for the case of treatment of bone cartilage complex, by using a tissue complex of an artificial
20 bone (e.g., hydroxyapatite construct such as NEO BONE, a microfibrous collagen medical device, etc.) and the synthetic tissue or complex of the present invention, it is possible to treat the bone by the artificial bone, and the cartilage on the bone by the synthetic tissue at the same time. It
25 is understood that the synthetic tissue or complex of the present invention is combined to an artificial bone and used for treatment. Herein, the implantable synthetic tissue or complex of the present invention substantially comprises, for example, cells and substances derived from the cells,
30 and more preferably, cells and extracellular matrix derived from the cells. The extracellular matrix as used herein is selected from the group consisting of collagen I, collagen III, vitronectin, and fibronectin.

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As used herein, the term "tissue complex" refers to a tissue obtained by combining a synthetic tissue or complex of the present invention with another synthetic tissue (including a synthetic tissue or complex of the present invention). Such a tissue complex can be used for a treatment of a plurality of tissues. For example, such a tissue complex can be used for treatment of both cartilage and bone.

In the case there is a large defect of soft tissue (e.g., meniscus, etc.), the synthetic tissue of the present invention can be coupled to another synthetic tissue (microfibrillar collagen medical device (e.g., CMI (Amgen, USA), Integran® (Nippon Zoki Pharmaceutical), hyaluronic acid gel, collagen gel, agarose gel, alginate gel, beads etc.) to promote biological integration between another synthetic tissue and an implantation cells.

Preferably, in the complex of the present invention, an implantable synthetic tissue and another synthetic tissue are biologically integrated. Such integration can be produced by culturing two tissues in contact. Such a biological integration is mediated by extracellular matrix.

Hereinafter, the present invention will be described by way of examples. Examples described below are provided only for illustrative purposes. Accordingly, the scope of the present invention is not limited except as by the appended claims.

(Examples)

In the examples below, animals were treated in

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accordance with rules defined by Osaka University (Japan) and were cared for in the spirit of animal protection.

(Example 1: Synovial cell)

5 In this example, various synovial cells were used to produce a synthetic tissue as follows.

<Preparation of cells>

10 Synovial cells were collected from a knee joint of a pig (LWD ternary hybrid, 2-3 months old upon removal of cells), followed by treatment with collagenase. The cells were cultured and subcultured in 10% FBS-DMEM medium (FBS was obtained from HyClone, DMEM was obtained from GIBCO). It has been reported that 10th passage synovial cells still
15 have pluripotency. Although cells of 10 or less passages were used in this example, cells of more than 10 passages may be used depending on the application. Autotransplantation was performed for humans, where a sufficient number of cells were used and the cells were
20 cultured for a short period of time so as to reduce the risk of infection or the like.

Considering these points, cells of various passages were used. Actually, primary culture cells, first passage
25 cells, second passage cells, third passage cells, fourth passage cells, fifth passage cells, sixth passage cells, eighth passage cells, and tenth passage cells were used in experiments. These cells were used for synthetic tissues.

30 <Preparation of synthetic tissue>

Synovial cells (4.0×10^6) were cultured in 2 ml of 10% FBS-DMEM medium in a 35-mm dish, a 60-mm dish, or 100-mm dish (SDBiosciences, culture dish and multiwell cell culture

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plate). In this case, ascorbic acid was added. The dishes, the ascorbic acid concentrations, and the cell concentration are described below.

5 Dishes: BD Biosciences, cell culture dishes and multiwell cell culture plates

Ascorbic acid 2-phosphate: 0 mM, 0.1 mM, 0.5 mM, 1 mM, 2 mM, and 5 mM

10

The number of cells: 5×10^4 cells/cm², 1×10^5 cells/cm², 2.5×10^5 cells/cm², 4.0×10^5 cells/cm², 5×10^5 cells/cm², 7.5×10^5 cells/cm², 1×10^6 cells/cm², 5×10^6 cells/cm², and 1×10^7 cells/cm²

15

Medium was exchanged two times per week until the end of a predetermined culture period. At the end of the culture period, a cell sheet was detached from the dish by pipetting circumferentially around the dish using a 100- μ l pipette man. After detachment, the cell sheet was made as flat as possible by lightly shaking the dish. Thereafter, 1 ml of medium was added to completely suspend the cell sheet. The cell sheet was allowed to stand for two hours, resulting in the contraction of the cell sheet into a three-dimensional form. Thus, a synthetic tissue was obtained (Fig. 1).

20

25

<Hematoxylin-Eosin (HE) Staining>

The acceptance or vanishment of cells in a sheet was observed by HE staining. The procedure is described as follows. A sample is optionally deparaffinized (e.g., with pure ethanol), followed by washing with water. The sample is immersed in Omni's hematoxylin for 10 min. Thereafter, the sample is washed with running water, followed by color

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development with ammonia in water for 30 sec. Thereafter, the sample is washed with running water for 5 min and is stained with eosin hydrochloride solution for 2 min, followed by dehydration, clearing, and mounting.

5

(Various extracellular matrix staining)

1. Make 5 μ m thick sections from frozen block.
2. Sections are fixed in acetone at -20°C for 5-10 mins.
(Paraffin blocks should be deparaffinized and
10 rehydrated).
3. Endogenous peroxide activity is blocked in 0.3% H_2O_2 in methanol for 20 mins at RT.
(1 ml 30% H_2O_2 + 99 ml methanol)
4. Wash with PBS (3 \times 5 mins).
- 15 5. Incubate with primary monoclonal antibody (a mouse or rabbit antibody against each extracellular matrix protein) in a moist chamber at 4°C for overnight (1 μ l antibody + 200 μ l PBS per slide).
6. Next day wash with PBS (3 \times 5 mins).
- 20 7. Apply anti mouse and anti rabbit no. 1 Biotynalated link for 30 mins -1 hrs at RT.
(apply about 3 drops directly on slide).
8. Wash with PBS (3 \times 5 mins).
9. Apply about 3 drops directly Streptavidin HRP no. 2 for
25 LSAB. 10-15 mins.
10. Wash with PBS (3 \times 5 mins).
11. Apply DAB (5 ml DAB+5 μ l H_2O_2).
12. Observe under microscope for brownish colour.
13. Dip in water for 5 mins.
- 30 14. Apply HE for 30 sec-1 min.
15. Wash several times.
16. Ion exchange water wash 1 time.
17. 80% ethanol wash for 1 min.

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18. 90% ethanol wash for 1 min.
19. 100% ethanol wash for 1 min (3 times).
20. Xylene wash for 1 min (3 times), Coverslip.
21. Examine color development.

5

An exemplary result is shown in Figure 1. As shown in the right portion of Figure 1, when ascorbic acid 2-phosphate was added as an ECM synthesis promoting agent, a contractile three-dimensional tissue of the cells was only slightly observed. On the other hand, by detaching the sheet-like cells from the base of the culture dish and allowing the cells to self organize, the cells were promoted to be layered and were accelerated into a three-dimensional structure, as shown in the left portion of Figure 1. As shown in a left portion of Figure 1, large tissue without a hole was also produced when synovial cells were used. This tissue was thick and its extracellular matrix was rich as shown in a right portion of Figure 1. When ascorbic acid 2-phosphate was added at a concentration of 0.1 mM or more, the formation of an extracellular matrix was promoted (Figure 2). Figure 3 shows an enlarged view of a synthetic tissue on Day 3, 7, 14, and 21. As can be seen, after 3 days of culture, the tissue was already so rigid that it can be detached (Figure 3). As the number of culture days is increased, the density of the extracellular matrix fluctuates and increases.

The tissue was detached from the base of the culture dish and self-contracted. The synthetic tissue was prepared in a sheet form. When the sheet was detached from the dish and was allowed to stand, the sheet self contracted into a three-dimensional structure. It is seen that a number of layers of cells exist in the tissue.

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Next, various markers including extracellular matrix markers were stained.

5 Figure 4 shows the result of staining extracellular matrix. It can be seen that various extracellular matrix components (collagen I, II, III, fibronectin, vitronectin, etc.) existed. Immunostaining was conducted, so that collagen I and III were strongly stained while collagen II
10 staining was limited to a portion. By being strongly magnified, it can be confirmed that collagen was stained at a site slightly away from the nuclei, i.e., collagen was a part of the extracellular matrix. On the other hand, fibronectin and vitronectin, which are believed to be
15 important cell adhesion molecules. By being strongly magnified, it can be confirmed that fibronectin and vitronectin were stained at a region close to nuclei unlike collagen, i.e., fibronectin and vitronectin existed around the cells.

20

These results demonstrated that cells of at least 3 to 8 passages are preferable for production of synthetic tissue.

25 For comparison, a normal tissue and a collagen sponge (CMI, Angen, USA) were stained. Figure 5 shows the normal tissue (normal synovial membrane tissue, tendon tissue, cartilage tissue, skin, and meniscus tissue). Figure 6
30 shows the stained collagen sponge, which was the comparative example. From the left, fibronectin, vitronectin, negative control, and HE staining are indicated. As can be seen, the conventional synthetic tissue was not stained with fibronectin or vitronectin. Therefore, the synthetic

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tissue of the present invention is different from conventional synthetic tissues. Existing collagen scaffolds do not contain fibronectin and vitronectin (adhesion agents). In view of this, the originality of the synthetic tissue of the present invention is clearly understood. No stain is found in the extracellular matrix. When the synthetic tissue of this example was compared with normal tissue, the synthetic tissue has a lower extracellular matrix density and had a structure different from normal tissue.

Further, when the synthetic tissue of the present invention was contacted with a filter paper in order to remove moisture from the tissues, the filter is adhered to the synthetic tissue, and it was difficult to manually detach the synthetic tissue of the present invention.

In order to determine the collagen concentration, the collagen content was measured. The result is shown in Figures 7 and 8. As can be seen, the amount of hydroxyproline clearly indicates that when 0.1 mM or more ascorbic acid 2-phosphate was added, the production of collagen was significantly promoted. The amount of produced collagen is substantially proportional to the time period of culture (Figure 8).

(Example 2: Measurement of collagen production)

Next, it was determined whether or not collagen (extracellular matrix) is sufficiently secreted after implantation of a synthetic tissue of the present invention. The following protocol was used.

<Method>

Culture periods: 3 days, 7 days, 14 days, and 21 days,

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Concentrations of ascorbic acid 2-phosphate: 0 mM, 0.1 mM, 1 mM, and 5 mM

Under the above-described conditions, a synovial membrane-derived synthetic tissue was produced.

5

6 N HCl was added to culture medium for the synthetic tissue, followed by hydrolysis at 105°C for 18 hours. The medium was oxidized with chloramine T. Thereafter, the synthetic tissue was subjected to color development using Ehrlich's Reagent Solution (2 g of p-dimethylamino-benzaldehyde + 3 ml of 60% perchloric acid; isopropanol was diluted at 3:13), followed by measurement of absorbance.

10

<Results>

1) The quantities of collagen produced was dependent on the ascorbic acid concentration in the following manner: 0 mM < 5 mM < 1 mM ≤ 0.1 mM (Figure 7 and 8).

15

2) it was demonstrated that the quantity of produced collagen is increased with an increase in the culture time period.

20

(Example 3: Influences of the size of a dish, the number of cells, and the number of passages)

Next, influences of the size of a dish and the number of passages were investigated.

25

Figure 9 shows the formation of synthetic tissues where the number of cells and the number of the passage were changed. A synthetic tissue was formed in all concentrations tested.

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Under the conditions of the above-described Example 1, a similar experiment was conducted where the sizes of dishes were 35 mm, 65 mm, and 100 mm and the number of passages were 5 to 7 (Figure 10).

5

The results are shown in Figures 9 and 10. Figure 9 shows the states of synthetic tissues, where the number of passages was changed. Figure 10 shows the states of synthetic tissues, where the size of a dish was changed. As can be seen from the figures, it was demonstrated that a synthetic tissue can be formed using any size of dish and any number of passages.

15

As shown in Figure 9, basically, a greater number of cells may be preferable for the purpose of matrix production. However, when an excessive number of cells were provided, the cells produced an excessive level of contraction force, so that the cell sheet was detached on the day following the start of culture. Therefore, it was demonstrated that when a larger synthetic tissue is desired, it is preferable to disseminate cells at a relatively small concentration. Particularly, in order to control the strength or the like of a synthetic tissue, a relatively small cell concentration seems to be preferable. As can be seen from the figure, when the number of passages was five, the resultant cell sheet was spontaneously detached if the cell concentration was $5.0 \times 10^5 / \text{cm}^2$, and was not spontaneously detached if the cell concentration was $2.5 \times 10^5 / \text{cm}^2$. Also, when the number of passages was six or more, the resultant cell sheet was spontaneously detached if the cell concentration was $7.5 \times 10^5 / \text{cm}^2$, and was not spontaneously detached if the cell

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concentration was $5.0 \times 10^5/\text{cm}^2$. Therefore, the production of a preferable synthetic tissue of the present invention seems to require a sufficient number of cells and a relatively great number of passages. Fourth passage cells were used to produce a trial synthetic tissue. It was spontaneously detached when the cell concentration was $40 \times 10^5/\text{cm}^2$. Thus, there seems to be a close relationship between the strength of a synthetic tissue and the number of passages. Various synthetic tissues can be produced, depending on the application. According to these results, cells capable of withstanding implantation seems to be obtained by culturing fifth passage cells at a concentration of $4.0 \times 10^5/\text{cm}^2$, however, the present invention seems not to be limited to this.

Similarly, the strength of tissues consisting of other cells is demonstrated to be able to be regulated by changing the cell concentration. Under the conditions described in Example 1, myoblasts can be used to produce a synthetic tissue and the influence of cell density on the strength of the synthetic tissue can be measured. Under the conditions described in Example 28, synovial cells can be used to produce a synthetic tissue and the influence of cell density on the strength of the synthetic tissue can be measured. Under the conditions described in Example 12, fat-derived cells can be used to produce a synthetic tissue and the influence of cell density on the strength of the synthetic tissue can be measured.

(Example 4: Measurement of mechanical properties)

In this example, cells (4×10^5 cells/ cm^2) were cultured in medium containing ascorbic acid 2-phosphate for three weeks. Following detachment at 48 hours, the mechanical properties of the tissue were investigated. The protocol

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will be described below.

The mechanical properties were examined by a tensile test.

5

Figures 11 and 12 show the outer appearance of a testing apparatus. Figure 11 shows a test piece holding portion (an original piece is shown). As shown in Figure 12, the opposite ends of a synthetic tissue were held by the test piece holding portion. A marker was attached to the synthetic tissue for ease of measurement. Figure 13 shows the attachment of the marker. Figure 14 shows an enlarged view of the test piece holding portion. Figure 15 shows the state of the synthetic tissue after a tensile test.

15

A synthetic tissue was held as shown in the figures and a marker was attached to the synthetic tissue, followed by a tensile test. The maximum load was 1.89 N, and the Young's modulus was 19.2 Mega pascal. As a reference, the maximum load (tension) of cartilage is typically 0.7 and that of skin is 1.2. The Young's modulus of cartilage is 10 MPa and that of skin is 35 Mpa. Thus, it was demonstrated that the synthetic tissue of the present invention has substantially the same mechanical strength as that of skin, cartilage, or the like, and can resist surgical handling.

25

The results of the experiment are shown in Figures 16 and 17. The results demonstrate that the maximum load was 1.89 N and 1.9 N, respectively. Young's modulus (tangent tensile modulus) was 19.2 MPa.

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(Example 5: Determination of self-supporting ability)

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Next, the self-supporting ability of a synthetic tissue of the present invention was tested. The synthetic tissue was held and tested using curved fine forceps A-11 (made of stainless steel; full length: 120 mm; curved: 20 mm, tip: 0.1 mm; manufactured by Natsume Seisakusho). It was determined by visual inspection whether or not the synthetic tissue has self-supporting ability. If the synthetic tissue was divided into a plurality of pieces, it was determined to lacking self-supporting ability. The same result was obtained when another forceps, e.g., curved fine forceps A-12-2 (made of stainless steel, full length: 100 mm; tip: 0.05 mm; manufactured by Natsume Seisakusho) were used by another experimenter performing the same experiment.

The self-supporting ability may be determined immediately after detaching a synthetic tissue off or after preserving a detached synthetic tissue.

None of the synthetic tissues comprising cardiomyocytes, myoblasts, and synovial cells, which are produced in the presence of a three-dimensional promoting agent comprising ascorbic acid as described in the above examples, had self-supporting ability. In contrast, it was already difficult to hold a synthetic tissue produced in the absence of such an agent with forceps upon detachment, so that lack of self-supporting ability was confirmed.

Therefore, 1) if a sheet is easily detached by circumferential pipetting; and 2) if the detached sheet is easily attached to a target site by lightly touching an edge thereof, the sheet spontaneously contracts to have sufficient strength.

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Therefore, self-supporting ability is a property which was first obtained by the method of the present invention.

5 (Example 6: Osteogenic differentiation induction)

In this example, it was determined whether or not the synthetic tissue of the present invention works when osteogenesis was induced in the synthetic tissue.

10 It was confirmed that synovial cells can be cultured in osteogenesis induction medium (10% FBS-DMEM+0.1 μ M dexamethasone, 10 mM β -glycerophosphate, 0.2 mM ascorbic acid 2-phosphate) from the beginning to produce a synthetic tissue.

15 Also, it was confirmed that a synthetic tissue was produced without osteogenesis induction, and thereafter, the medium was exchanged with osteogenesis induction medium and the tissue was cultured, so that calcified bone was generated in the synthetic tissue. The result is shown in Figure 18.

25 Whereas a synthetic tissue without differentiation induction appears to be transparent, an ossified synthetic tissue has a white colour. The synthetic tissue was strongly stained with Alizarin Red, and was also strongly stained by alkali phosphatase (ALP) staining as compared to the control. Thus, it was confirmed that the synthetic tissue of synovial cells is capable of osteogenesis.

30 (Example 7: chondrogenesis induction)

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In this example, it was determined whether or not chondrogenesis induction can be used for the production method of the synthetic tissue of the present invention.

5 (Culture conditions)
Cell density: 4×10^4 cells/cm²
Conditions: CO₂ 5%, air 95%, 37°C

10 These conditions and a chondrogenesis induction medium described below were used to produce a synthetic tissue.

15 Cartilage differentiation induction medium: DMEM (GIBCO), FBS (HyClone) 10%, ITS+Premix (insulin, transferrin, selenious acid) (BD Biosciences) 6.25 µg/ml, dexamethasone (Sigma) 10^{-7} M, ascorbic acid (Wako) 50 µg/ml, pyruvic acid (SIGMA) 100 µg/ml.

20 The results are shown in Figure 19. The cells were induced into cartilage. From the left, a typical medium, a chondrogenesis induction medium, a chondrogenesis induction medium+BMP-2, and a chondrogenesis induction medium+TGF-β1 were used to culture a synthetic tissue. All of the tissues were stained blue with Alcian blue staining.
25 It was confirmed that a cartilage-like matrix production was accelerated. Such an effect is significant for cells cultured in medium containing BMP-2. The result of quantification of staining ability is shown in Figure 20.

30 Expression of cartilage-associated genes (aggrecan, Col II, Sox9) in the synthetic tissue is shown in Figure 21. When the synthetic tissue was transferred from the typical medium (leftmost column) to the chondrogenesis induction

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medium (middle column), expression of the Sox9 gene, which is a chondrogenesis marker, was increased. When the synthetic tissue was further cultured in the chondrogenesis induction medium+BMP-2, expression of the collagen II gene was also increased. Thus, stronger chondrogenesis could be confirmed. Figure 22 shows the results of comparison of a chondrogenesis reaction between a monolayer culture synovial cell and a synovial cell in a three-dimensional synthetic tissue, when the same differentiation inducing stimulus was applied. When counted from the left, odd-numbered columns indicate monolayer culture, while even-numbered columns indicate three-dimensional synthetic tissue, where culture was performed under the same culture conditions. When the chondrogenesis induction medium or the chondrogenesis induction medium+BMP-2 was added as a stimulus, it was confirmed that the chondrogenesis marker gene was significantly expressed in the synthetic tissue. Thus, the three-dimensional synthetic tissue was confirmed to have strong chondrogenesis ability.

20

(Example 8: Repair of a pig cartilage)

Next, it was determined whether or not cartilage can be repaired. An allogenic synthetic tissue was used.

25

To determine the presence or absence of the adhesion capability of a synthetic tissue, an allogenic synthetic tissue was implanted onto a pig cartilage piece. The synthetic tissue was prepared under conditions where the number of cells was 4.0×10^5 cells/35-mm dish, the concentration of ascorbic acid was 1 mM, and the culture period was 7 to 14 days. A wound having a diameter of 6 mm was generated on the cartilage piece. An upper layer zone thereof was cut off from the cartilage piece using a scalpel.

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Chondroitinase ABC (1 U/ml) was added. The cartilage piece was allowed to react for 5 minutes. A synthetic tissue was sized to have a diameter of 6 mm and was implanted, followed by culture for 7 days. The synthetic tissue is closely attached to the attachment surface of the cartilage piece. Fibronectin aggregated on the attachment surface (Figure 23).

Next, pig cartilage implantation was performed. As described above, a wound having a diameter of 6 mm was created in a medial femoral condyle. An upper layer zone thereof was cut off from the cartilage piece using a scalpel. Chondroitinase ABC (1 U/ml) was added. The cartilage piece was allowed to react for 5 minutes. A allogenic synthetic tissue was sized to have a diameter of 6 mm and was implanted, followed by culture for 7 days. The results are shown in Figure 24. Figure 25 shows a strongly enlarged view of a culture portion of a surface of the cartilage adhered to the synthetic tissue of Figure 24. The left portion of Figure 25 is a photograph showing the result of HE staining, the middle portion is a photograph showing the result of staining with anti-fibronectin antibodies, and the right portion is a photograph showing the result of staining with anti-vitronectin antibodies. As indicated by an arrow (the interface between the synthetic tissue and the cartilage tissue), it was demonstrated that the matrix of the synthetic tissue was directly attached to the cartilage matrix, but not via cells. It is shown that fibronectin and vitronectin were accumulated at the adhesion surface. Thus, the results suggest that these adhesion molecules are involved in adhesion between a synthetic tissue and a recipient tissue. Therefore, the present invention is also characterized in that the synthetic tissue is more effectively adhered to

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in vivo tissue than conventional synthetic tissues, or cells.

Further, the tissue was examined after one month of implantation. The result is shown in Figure 26. As can be seen, it is confirmed that the synthetic tissue was biologically integrated with the cartilage injury portion and was accepted without inflammation. The surface layer portion of the synthetic tissue was made mainly of fibroblast-like cells as shown in Figure 27. On the other hand, a deeper layer portion of the synthetic tissue was made mainly of cartilage-like cells as shown in Figure 28. Therefore, the implanted synthetic tissue had differentiated into cartilage-like tissue over time. No significant rejection was confirmed in any period of time, and rejection which is expected for allogenic implantation, was not observed.

Therefore, it was found that the allogenic synthetic tissue can be implanted without a side effect.

20

(Example 9: Repair of a pig meniscus)

Next, it was determined whether or not the synthetic tissue of the present invention is applicable to repair of meniscus.

25

As in the above-described Example 6, an allogenic synthetic tissue was prepared under conditions where the number of cells was 4.0×10^6 cells/35-mm dish, the concentration of ascorbic acid was 1 mM, and the culture period of time was 7 to 14 days. A portion having a diameter of 6.5 mm was removed from a meniscus (Figure 29), and the synthetic tissue was implanted thereinto. The portion containing the implant was covered with a collagen sheet

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(Nipro) for protection until the synthetic tissue was accepted (Figure 30). The pig was kept for one month. The protocol is described below.

5 (Anesthesia)

A pig 15 to 17 weeks old (LWD ternary hybrid) was intramuscularly injected via the dorsal portion of its neck with 20 mg/kg Ketalar + 10 mg/kg Seralactal. Thereafter, an infusion route was provided in the ear vein, and thereafter, 10 the respiratory tract was secured using endotracheal intubation. Diprivan was continuously administered at a rate of 0.5 mg/kg/hr to maintain anesthesia. An antibiotic (Cefamezin, 1 g) was administered to prevent post-operational infection.

15 (Operation)

The animal was positioned and an operation portion was cleaned with a sterilized drape. A knee joint was accessed by medial para-patellar approach. After detecting 20 the internal articular capsule, the middle portion at the medial collateral ligament (MCL) of the knee was defected. A cylinder-shaped cavity (diameter: 6.5 mm) was created using the mosaic plasty DP (Smith & Nephew) (Figure 29). The cavity was filled with the synthetic tissue (Figure 30), followed 25 by the coverage with fascia. After hemostasis was confirmed, the incised internal collateral ligament was repaired, and the articular capsule, the subcutaneous tissue, and the epidermis were sutured. A cast was fixed to the knee joint in its incurvation position. The operation was ended.

30 (Evaluation method)

Visual inspection and histological study were performed.

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(Results)

Four weeks after operation, the animals receiving the synthetic tissue was significantly repaired according to visual finding (Figure 31) and histological finding (Figure 32).

Remarkably, an eosin positive result was observed in the synthetic tissue four weeks after implantation. Also, the formation of a meniscus tissue-like matrix was observed and the biological integration of the synthetic tissue and its adjacent meniscus tissue was completed.

(Example 10: Repair of pig tendon/ligament tissues)

Tendon/ligament tissues were subjected to a repair operation. The state of the wound of a tendon/ligament tissue is confirmed. In this case, a portion of synovial cells are collected. The synovial cells are cultured. The cells are used to produce a synthetic tissue using a protocol as described in Example 1.

Next, by operation, the vicinity of the wound site of the tendon/ligament tissue is cut off to obtain a fresh portion, on which the above-described synthetic tissue is in turn placed. In this case, since the synthetic tissue has adhesion molecules, the synthetic tissue is adhered to the portion without suture. The protocol is described below.

(Anesthesia)

A pig 15 to 17 weeks old (LWD ternary hybrid) was intramuscularly injected via the dorsal portion of its neck with 20 mg/kg Ketalar + 10 mg/kg Seractal. Thereafter, an infusion route was provided in the ear vein, and thereafter,

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the respiratory tract was secured using endotracheal intubation. Diprivan was continuously administered at a rate of 0.5 mg/kg/hr to maintain anesthesia. An antibiotic (Cefamezin, 1 g) was administered to prevent post-operational infection.

(Operation)

The animal was positioned and an operation portion was cleaned with a sterilized drape. A knee joint was accessed by medial para-patellar approach. After detecting the internal articular capsule, the middle portion of the capsule was dissected. The lower thighs were bent and laterally rotated, and were further pulled forward, so that the anterior horn portion of the internal meniscus was exposed. In this place, a cylinder-shaped cavity (diameter: 6.5 mm) was created using the mosaic plasty DP (Smith & Nephew). The cavity was filled with the synthetic tissue. In order to protect the synthetic tissue until it was accepted, the meniscus was wrapped with a collagen sheet (Nipro) which was fixed by suture. After hemostasis was confirmed, the incised internal collateral ligament was repaired, and the articular capsule, the subcutaneous tissue, and the epidermis were sutured. A cast was fixed to the knee joint in its incurvation position. The operation was ended.

(Evaluation method)

Histological study was performed based on Frank's method (J. Orthop. Res., 13, 923-9, 1995).

(Results)

According to visual finding and histological finding 6 weeks after operation, the group filled with the synthetic tissue had significantly better healing quality.

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(Example 11: Repair of a pig bone)

In this example, repair of bone is experimentally conducted. Using a protocol as described in Example 1, synovial cells are collected and cultured to produce a synthetic tissue.

Next, a sheet of this synthetic tissue is applied to a bone. The synthetic tissue is applied to an affected portion mainly by covering it over a cortical bone as well as a periosteum. As a result, it is demonstrated that the synthetic tissue comprising synovial cells is effective for repair of a bone. The protocol is described below.

(Anesthesia)

A pig 15 to 17 weeks old (LWD ternary hybrid) was intramuscularly injected via the dorsal portion of its neck with 20 mg/kg Ketalar + 10 mg/kg seractal. Thereafter, an infusion route was provided in the ear vein, and thereafter, the respiratory tract was secured using endotracheal intubation. Diprivan was continuously administered at a rate of 0.5 mg/kg/hr to maintain anesthesia. An antibiotic (Cefamezin, 1 g) was administered to prevent post-operational infection.

25

(Operation)

The animal was positioned and an operation portion was cleaned with a sterilized drape. A second metatarsal bone was accessed from a longitudinal incised portion. The periosteum of the second metatarsal bone was ablated as much as possible so that the surface of the second metatarsal bone was exposed. A window of 1.5 cm (horizontal) x 3 cm (vertical) was created on the surface of the second metatarsal

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bone using a chisel. The window was covered with the
outstretched synthetic tissue. After confirming the
attachment of the synthetic tissue, the the subcutaneous
tissue and the epidermis were sutured. A cast is fixed to
5 the lower thigh. The operation was ended.

(Evaluation method)

Radiography, micro CT, and histology.

10 (Results)

Four weeks after operation, evaluation confirmed
that osteogenesis was accelerated in the window portion for
the group filled with the synthetic tissue.

15 (Example 12: Pig fat-derived tissue)

Next, cells derived from adipose tissue were used
to produce a synthetic tissue.

A) Cells were collected as follows.

20 1) A specimen was removed from the fat-pad of a knee
joint.

2) The specimen was washed with PBS.

3) The specimen was cut into as many pieces as
possible using scissors.

25 4) 10 ml of collagenase (0.1%) was added to the
specimen, followed by shaking for one hour in a water bath
at 37°C.

5) An equal amount of DMEM (supplement with 10% FBS)
was added, followed by filtration using a 70 µl filter
30 (available from Millipore or the like).

6) Cells which passed through the filter and residues
which remained on the filter were placed in a 25-cm² flask
(available from Falcon or the like) containing 5 ml of DMEM

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supplemented with 10% FBS.

7) Cells attached to the bottom of the flask (including mesenchymal stem cells) were removed and subjected to the production of a synthetic tissue as follows.

5

B) Production of synthetic tissue

Next, the above-described fat-derived cells were used to produce a synthetic tissue. The concentrations of ascorbic acid 2-phosphate were 0 mM (absent), 0.1 mM, 0.5 mM, 10 1.0 mM, and 5.0 mM. The synthetic tissue was produced in accordance with the above-described method which was used to produce synovial cells (Example 1). Cells were disseminated at an initial concentration of 5×10^4 cells/cm². The result is shown in Figure 33. The cells were cultured for 14 days. 15 A synthetic tissue was also formed from an adipose tissue-derived cell and had as rich fibronectin and vitronectin as the synovial cell-derived synthetic tissue. Collagen I and III were similarly expressed richly.

20

C) Implantation experiment

Next, the above-described synthetic tissue is subjected to an implantation experiment in Example 8 25 (cartilage repair) and in Example 9 (meniscus repair). As a result, it is demonstrated that a repairing capability is possessed by the fat-derived synthetic tissue as with a synovial cell-derived synthetic tissue.

30

D) Differentiation induction of a fat-derived synthetic tissue into bone/cartilage

The synthetic tissue of this example was induced to differentiate into a cartilage or a bone. The results

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are shown in Figure 34. The left portion of the figure indicates the results of an osteogenesis experiment. The upper portion indicates a synthetic tissue, while the lower portion indicates monolayer culture. The synthetic tissue had a positive reaction to Alizarin Red in an osteogenesis induction medium. Thus, osteogenesis was confirmed. The right portion indicates a chondrogenesis induction experiment. In this experiment, the synthetic tissue was differentiated with a stimulus due to chondrogenesis induction medium+BMP-2 into a cartilage-like tissue which was positive to Alcian blue. Thus, it was demonstrated that the fat-derived synthetic tissue also has the ability to differentiate into a bone and a cartilage as with a synovial cell-derived synthetic tissue.

(Example 13: Versatility of shape of synthetic tissue)

In this example, a difference in function due to the shape of a synthetic tissue is measured. The synthetic tissue may be crumpled up and implanted into an affected portion instead of using a sheet of the synthetic tissue. Thereby, it is determined whether or not a tailor-made operation can be conducted, depending on the shape or the like of a wound portion.

In this example, it is investigated whether or not a synthetic tissue can be implanted when it is in the shape of a ball, a line, or a tube. The synthetic tissue is confirmed not to require suture, since it has an adhesion molecule.

(Example 14: Treatment using a synovial cell)

In this example, a synovial cell is collected from a patient having an injured meniscus, and it is determined

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whether or not the synovial cell can be used to produce a synthetic tissue.

(Collection of a human synovial cell)

5 A human patient, who has a clinical symptom is diagnosed by an imaging technique as having cartilage injury or meniscus injury, is subjected to arthroscopy under lumbar anesthesia or general anesthesia. In this case, several milligrams of synovial membrane is collected. The collected
10 synovial membrane is transferred to a 50-ml centrifuge tube (manufactured by Falcon) and washed with phosphate buffered saline (PBS). Thereafter, the sample is transferred to a 10-cm diameter culture dish (Falcon) and is cut into small pieces using a sterilized blade. Thereafter, 10 ml of 0.1%
15 collagenase (Sigma) is added to the cut pieces in the dish. The dish is shaken in a constant temperature bath at 37°C for 1 hour 30 minutes. To the solution, 10 ml of medium (DMEM, Gibco) containing self-serum previously collected or bovine serum (FBS) is added to inactivate the collagenase, followed
20 by centrifugation at 1500 rpm for 5 minutes to pellet the cells. Thereafter, 5 ml of the serum-containing medium is added again. The culture medium is passed through a 70- μ l filter (Falcon). The collected cells are transferred to a 25 cm² flask (Falcon), followed by culture in a CO₂ incubator
25 at 37°C.

(Subculture of a synovial cell)

During primary culture, medium is exchanged two times every week. When cells become confluent, the cells are
30 subcultured. For initial subculture, the medium is suctioned and thereafter the cells are washed with PBS. Trypsin-EDTA (Gibco) is added to the cells which are in turn allowed to stand for 5 minutes. Thereafter, the

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serum-containing medium is added and the resultant mixture was transferred to a 50-ml centrifuge tube (Falcon), followed by centrifugation at 1500 rpm for 5 minutes. Thereafter, 15 ml of the serum-containing medium is added to the pellet.

5 The cells are placed in a 150-cm² culture dish (Falcon). Subsequent subculture is performed so that the cell ratio was 1:3. The same procedure is repeated up to 4 to 5 passages.

(Production of a synthetic tissue)

10 The synovial cell of 4 to 5 passages is treated with trypsin-EDTA. The synovial cells (4.0×10^6) are dispersed in 2 ml of medium containing 0.2 mM ascorbic acid 2-phosphate on a 35-ml culture dish (Falcon), followed by culture in a CO₂ incubator at 37°C for 7 days. As a result, a culture

15 cell-extracellular matrix complex is formed. The complex is mechanically detached from the culture dish by pipetting the periphery thereof two or more hours before an implantation operation. After detachment, the culture cell-extracellular matrix complex contracts into a

20 three-dimensional tissue having a diameter of about 15 mm and a thickness of about 0.1 mm.

(Example 15: Production of a synthetic tissue from a human adipocyte)

25 A collection-intended site (e.g., around a knee joint) from a patient under local anesthesia is resected. Several milligrams of adipocytes are collected from the site. The collected adipocytes were treated in a manner similar to that of the synovial cells. As a result, a

30 three-dimensional synthetic tissue can be produced.

(Example 16: Implantation of a synthetic tissue into a joint cartilage injury portion)

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The synthetic tissue produced in Example 14 or 15 is used for actual implantation. A human subject is subjected to lumbar anesthesia or general anesthesia. Thereafter, the inside of a joint is opened at a minimum incision for arthroscopy.

5 After detecting a cartilage injury portion, the size of the cartilage injury is measured. A circular portion of the cartilage is dissected from the bone-cartilage interface using the mosaic plasty harvesting system (Smith and Nephew) and a dental explorer, where the circular portion fully

10 contains the injured cartilage. The synthetic tissue was implanted into the cavity in a portion of cartilage. The synthetic tissue is adhered to the base of the cavity several minutes after implantation. When an affected portion receives a high mechanical stress, the fixation of the

15 synthetic tissue may be reinforced using fibrin glue (initial fixation is reinforced). The present invention is not limited to this. After fixation, the articular capsule, the subcutaneous tissue, and the skin are sutured collectively. After closing the incision site, the joint is fixed using

20 a cast or an orthosis for 2 to 3 weeks. Thereafter, rehabilitation is started within a limited range of motion. When an affected portion is present in a weight-bearing joint (e.g., a knee, a ankle joint, etc.). A full load is able to be applied after 6 to 8 weeks.

25

As a result, symptoms are cured or ameliorated as follows: a reduction in joint pain when a load or an exercise is applied; elimination of joint effusion; recovery of a joint range of motion; recovery of muscle strength around

30 the joint; prevention of osteoarthritis; and the like. Thus, it is observed that the synthetic tissue of the present invention has no significant side effects and improves the function of a repaired portion.

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(Example 17: Implantation into a meniscus injury portion)

5 In this example, the synthetic tissue produced in Example 14 or 15 is actually implanted into a meniscus injury portion.

10 A meniscus injury portion is detected in a human subject under lumbar anesthesia or general anesthesia, using an arthroscope. A rupture portion of an injury meniscus is filled with the synthetic tissue. Thereafter, the injured meniscus and the synthetic tissue are sutured together. All surgical procedures are performed under an arthroscope. After surgery, a knee orthosis is used for 2 to 3 weeks.
15 Thereafter, rehabilitation is started within a limited range of motion. A full weight bearing is permitted after 5 to 6 weeks.

20 As a result, symptoms are cured or ameliorated as follows: a reduction in joint pain when a load or an exercise is applied to the knee joint; elimination of hydrarthrosis; recovery of a joint range of motion; recovery of muscle strength around the joint; recovery of activity; doing sports again; and the like. Thus, it is observed that the synthetic
25 tissue of the present invention has no significant side effects and improves the function of a repaired portion.

(Example 18: Implantation into an Achilles tendon)

30 The synthetic tissue produced in Example 14 or 15 is implanted into an Achilles tendon injury portion.

A human subject under lumbar anesthesia or general anesthesia is subjected to Achilles tendon by para-tendon

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approach. The portion of degenerative tear is detected and then curetted. The synthetic tissue is implanted into the portion of degenerative tear. After implantation, conventional tendon repair is performed. In addition, the surface layer of the repaired portion is covered with the synthetic tissue, which is in turn sutured and fixed thereto. After closing the incision site, a cast is fixed to the lower limb for 4 weeks. A full weight bearing is permitted after 6 to 8 weeks.

As a result, symptoms are cured or ameliorated as follows: recovery of activity level (from walking to a sport level); a reduction in pain; and a decrease in possibility of re-rupture. Thus, it is observed that the synthetic tissue of the present invention has no significant side effects and improves the function of a repaired portion.

(Example 19: Treatment of intractable pseudarthrosis)

In this example, intractable pseudarthrosis is treated using the synthetic tissue produced in Example 14 or 15. A feature of intractable pseudarthrosis is that a periosteum, which is a source of supplying cells in a bone fracture therapy, is severely damaged and lost. Implantation of the synthetic tissue is considered to be appropriate in such a case.

A bone fracture portion is opened in a human subject under anesthesia. Thereafter, the bone fracture portion is curetted. After the remaining portion is fixed with a plate or an intramedullary nail, the injured periosteum is covered with the synthetic tissue. The synthetic tissue is sutured and fixed to adjacent periosteum tissue. After closing the

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incision site, the joint adjacent to the bone fracture portion is fixed with a cast for 3 to 4 weeks. In the case of a lower limb bone, full weight bearing is permitted after 6 to 8 weeks.

5

As a result, symptoms are cured or ameliorated as follows: elimination of pain; recovery of muscle strength around the joint; and recovery of an activity level. Thus, it is observed that the synthetic tissue of the present invention has no significant side effects and improves the function of a repaired portion.

10

(Example 20: Implantation into a rotator cuff injury portion)

15

In this example, a synthetic tissue is implanted into a rotator cuff injury portion. The synthetic tissue is produced as described in Example 1. Under general anesthesia, the rotator cuff injury portion is detected by transdeltoid approach.

20

After detecting the rotator cuff injury portion, the portion is curetted and is subjected to a typical rotator cuff repair operation. Thereafter, the surface layer of the repaired rotator cuff portion is covered with the synthetic tissue. After closing the incision site, the shoulder joint is fixed with an orthosis for 2 to 3 weeks. Thereafter, rehabilitation is started within a limited range of motion. After 6 weeks, full range of motion is permitted.

25

30

As a result, symptoms are cured or ameliorated as follows: remission of shoulder pain (particularly, night pain); recovery of a joint range of motion; recovery of muscle strength around the shoulder; and recovery of activity. Thus,

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it is observed that the synthetic tissue of the present invention has no significant side effects and improves the function of a repaired portion.

5 (Example 21: Study on the possibility of cell differentiation induction before and after production of a synthetic tissue)

In this example, a synthetic tissue is produced using a human synovial cell.

10

The production process of the synthetic tissue using a human synovial cell is shown in the upper portions of Figures 35 and 36. Figure 35 shows production of a synthetic tissue after a human synovial cell is subjected to differentiation induction. Figure 36 shows that a synthetic tissue is produced before the tissue is subjected to differentiation induction. The differentiation induction is performed by culturing a human synovial cell in DMEM medium containing 0.1 μ M dexamethasone, 10 mM β -glycerophosphate, and 50 μ g/ml ascorbic acid 2-phosphate for 14 days. The synthetic tissue is stained with Alizarin red and alkaline phosphatase (ALP). The results of the staining are shown in the lower portions of Figures 35 and 36. As can be seen from Figure 35, in either case, the synthetic tissue is produced and exhibits an osteogenic reaction positive to the Alizarin red and ALP staining. Therefore, it is demonstrated that the differentiation induction of a tissue can be performed either before or after production of a synthetic tissue.

25
30

(Example 22: Study on timing of differentiation for production of a synthetic tissue in the case of human cells)

In this example, a synthetic tissue was produced using

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cells derived from adipose tissue.

A) The cells were collected as follows.

1) A specimen was collected from a fat-pad of a knee
5 joint.

2) The specimen was washed with FBS.

3) The specimen was cut into as many pieces as
possible.

4) 10 ml of collagenase (0.1%) was added, followed
10 by shaking in 37°C water bath for one hour.

5) An equal amount of DMEM (supplemented with 10%
FBS) was added. The resultant mixture was passed through
a 70- μ l filter (available from Millipore, etc.).

6) Cells passing through the filter and cells
15 remaining on the filter were cultured in 25-cm² flask
containing 5 ml of DMEM medium supplemented with 10% FBS.

7) The cells (including a mesenchymal stem cell)
attached to the base of the flask were used to produce a
synthetic tissue as follows.

20

B) Production of a synthetic tissue

Next, the fat-derived cells were used to produce a
synthetic tissue. Ascorbic acid 2-phosphate was used at a
concentration of 0 mM (absence), 0.1 mM, 0.5 mM, 1.0 mM,
25 or 5.0 mM. The production was conducted in accordance with
the method for producing a synthetic tissue from a synovial
cells (Example 1). The cells were disseminated at an initial
density of 5×10^4 cells/cm².

30

The cells were used to study the importance of the
differentiation timing using the conditions as described
in Example 21.

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As a result, it was similarly demonstrated that the differentiation timing has no particular influence on the adipocyte-derived synthetic tissue of the present invention.

5 (Example 23: Confirmation of biological integration)

It is known that conventional collagen gel does not always achieve biological integration after implantation. In this example, a conventional collagen gel (3% type I collagen, Koken, Tokyo, Japan) was used. Synovial cells (1×10^5 cells/ml) were embedded in the gel. The resultant gel was implanted into a cavity in a portion of cartilage. As a result, as can be seen from Figure 37, the integration between the collagen gel and its adjacent cartilage was insufficient, so that a crack was observed (arrow in Fig 37).

On the other hand, when a synthetic tissue of the present invention as produced in Example 1 is introduced into a pig, biological integration is histologically established as shown in Figure 38.

25 (Example 24: Study on conditions for detachment during production of a synthetic tissue)

In this example, it was determined whether or not chemical detachment can be used instead of physical detachment (mechanical detachment (e.g., pipetting, etc.)) during the production of the synthetic tissue of the present invention.

30

(Conditions for culture)

Cell density: 4×10^4 cells/cm²

Conditions: CO₂ 5%, air 95%, 37°C

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Medium: DMEM/F12 (FBS 10%) supplemented with
10 ng/ml TGF β 1.

5 This medium was used to conduct culture under the
conditions described in Examples 14 and 15 to produce a
synthetic tissue.

10 When TGF- β was added, the monolayer culture cells
could be more easily detached from the culture dish.

15 Medium: DMEM (GIBCO), FBS (HyClone) 10%, ITS+Premix
(insulin, transferrin, selenious acid) (BD Biosciences)
6.25 μ g/ml, dexamethasone (Sigma) 10^{-7} M, ascorbic acid
(WAKO) 50 μ g/ml, pyruvic acid (SIGMA) 100 μ g/ml.

20 The results are shown in Figures 19 and 39. The
rightmost column in Figure 19 shows the case where TGF- β
was added. In this case, cells were detached from a culture
dish during monolayer culture. Therefore, a synthetic
tissue could not be satisfactorily produced. Figure 39
shows the result of a tissue which was detached without a
physical stimulus when TGF- β was added in monolayer culture.
These results indicate that TGF- β has the effect of detaching
culture cells.

25 (Example 25: Actin regulatory agent)

30 Dihydrocytochalasin B and YZ7632 (Yamanouchi
Pharmaceutical), which are known to have an actin
depolymerizing function, were used to study their influence
on the contraction of a synthetic tissue.

A synovium-derived synthetic tissue was produced by
monolayer culture. The tissue was detached from a culture

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dish. The tissue was cultured in medium in the presence of dihydrocytochalasin B (3 μ M) and Y27632 (10 μ M). The transition of the radius of the tissue is shown every unit culture time in Figure 40. As can be seen from the figure, contraction was inhibited by the addition of these actin depolymerizing agents. Dihydrocytochalasin B and Y27632 are representative exemplary actin polymerization inhibitors. It will be understood by those skilled in the art that other actin polymerization inhibitors, such as cytochalasin D and the like, have a similar function.

(Example 26: Production of an artificial bone/cartilage column as a complex of a synthetic tissue and an artificial bone)

A 12-well culture dish was used to produce a synthetic tissue.

A column-like artificial bone (NEO BONE: MMT) having a diameter of 5 mm \times 6 mm was placed in a 96-well culture dish. The synthetic tissue was implanted onto the artificial bone. 100 μ l of medium (DMEM, 10% FBS) was placed in each well of the dish, followed by culture for 2 hours. As a result, the synthetic tissue was attached to the artificial bone, thereby obtaining a tissue complex.

This complex was cultured in cartilage induction medium (DMEM, 10% FBS, ITS+Premix, sodium pyruvate, ascorbic acid 2-phosphate, 500 ng/ml BMP-2) for 14 days.

The result is shown in Figure 41.

As can be seen from Figure 41, it is demonstrated that the synthetic tissue of the present invention was

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satisfactorily adhered to the other synthetic tissue (i.e., the artificial bone). Therefore, it will be understood that the synthetic tissue of the present invention can be combined with other synthetic tissues into a tissue complex.

5

(Example 27: Composite tissue obtained by attaching a synthetic tissue to a collagen scaffold)

In this example, a microfibrinous collagen medical device (specifically, a collagen synthetic tissue (CMI (Collagen Meniscal Implant) collagen sponge, Amgen, USA)) was attached to a synthetic tissue instead of NEO BONE in Example 26. The result is shown in Figure 42 (enlarged photograph). The synthetic tissue of the present invention is observed to be biologically integrated with the surface of the CMI. Thus, it was demonstrated that a microfibrinous collagen medical device, which is a conventional synthetic tissue, can be combined with the synthetic tissue of the present invention to obtain a tissue complex.

20

(Example 28: Production of a synthetic tissue using a myoblast)

In this example, an influence of ascorbic acid or a derivative thereof on the production of a synthetic tissue when a myoblast was used, was studied. The synthetic tissue was produced as in Example 1.

25

After the myoblast was well grown, 5×10^6 myoblast cells were cultured to form a synthetic tissue. For culture, SkGM Basal Medium (Clonetics (Cambrex)) was used. Next, ascorbic acid 2-phosphate (0.5 mM), a magnesium salt of ascorbic acid 1-phosphate (0.1 mM), and L-ascorbic acid Na (0.1 mM) were added to the medium. After four days of culture, the tissue was detached. As a control, a synthetic tissue

30

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was produced in medium without ascorbic acids.

(Results)

When ascorbic acids were used, the synthetic tissue
5 was easily detached as compared to when the ascorbic acid-free
culture system was used. Also, in the ascorbic acid-free
culture system, the tissue was cultured to about several
millimeters. When the tissue exceeded such a level, a crack
or the like occurred in the tissue so that the tissue did
10 not grow satisfactorily. In addition, it was substantially
difficult to detach the tissue. Thus, no implantable
synthetic tissue was produced (Figure 43). In contrast, the
synthetic tissue of the present invention, which was cultured
in medium containing ascorbic acids, was grown to a size
15 which allows implantation, and was easily isolated
(Figure 44). Biological integration was investigated, so
that extracellular matrices were highly interacted
(Figure 45).

20 (Example 29: Effect of a synthetic tissue in the
presence of ascorbic acids)

The synthetic tissue of Example 28, which was
produced in the presence of ascorbic acids, was implanted
into a dilated cardiomyopathy rat. In 28 rats, the left
25 anterior descending (LAD) was ligated for two weeks to produce
injured hearts. The synthetic tissue of the present
invention was implanted into some of the injured hearts,
while the synthetic tissue of the present invention was not
implanted into the other injured hearts. As controls, rats
30 without injury to their hearts were obtained.

The rats were anesthetized and operated. The heart
function of the rats was monitored on Day 14 and 28 after

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5 surgery. A ultrasonic instrument (Sonos 5500) having an
anular array converter operating at 12 MHz was used to perform
endocardiography. Parasternal minor axis imaging and
parasternal major axis imaging were performed in a B-imaging
mode and an M-imaging mode. In addition to the anterior wall
pressure, general parameters (e.g., left ventricular
telediastolic diameter, left ventricular telesystolic
diameter, internal diameter contraction rate, and ejection
fraction) were measured.

10

Two and four weeks after implantation, the rats were
sacrificed with an excessive amount of pentobarbital. The
heart was dissected, fixed with 10% formalin, and embedded
in paraffin. In a low temperature bath, the heart was cut
15 along the longitudinal axis thereof from the base to the
apex to prepare a series of sections having a thickness of
5 mm. Thereafter, the sections were treated for standard
histology.

20

All of the rats with implants were completely cured,
and survived for substantially the same period of time as
normal rats. Therefore, it was demonstrated that the present
invention can completely cure diseases, which are
conventionally said to be intractable, in the presence of
25 a specific ECM synthesis promoting agent.

(Example 30: Combined therapy)

A combined therapy of the synthetic tissue produced
in the examples and a gene therapy was performed. The
combined therapy was intended to promote vascularization
30 in a portion which a synthetic tissue was implanted; promotion
of acceptance of an implanted synthetic tissue; and
suppression of cell necrosis in a synthetic tissue.

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(Methods)

A hemagglutinating virus of Japan (HVJ)-liposome complex was prepared in accordance with Kaneda Y., Iwai K., Uchida T., Increased expression of DNA co-introduced with nuclear protein in adult rat liver. Science, 1989;243:375-378. The procedure will be briefly described below. A DNA solution (200 μ l) was added, followed by shaking for 30 seconds. The solution was allowed to stand at 37°C in a constant temperature bath for 30 seconds. This step was performed 8 times. Thereafter, ultrasonication was performed for 5 seconds, followed by shaking for 30 seconds. BSS (0.3 ml) was added, followed by shaking at 37°C in a constant temperature bath. Inactivated HVJ was added. The mixture was placed on ice for 10 minutes. The mixture was then shaken at 37°C in a constant temperature bath for one hour. A 60% sucrose solution (1 ml) and a 30% sucrose solution (6 ml) were layered in a centrifuge tube. A HVJ liposome solution was placed on top of the layered sucrose solution. Additional BSS was added to the tube. Centrifugation was performed at 62,800 g at 4°C for 1.5 hours. A solution immediately above the 30% sucrose solution layer was recovered. The solution was preserved at 4°C and was used for gene introduction.

About 0.2 ml of Sendai virus liposome-plasmid complex (including 15 μ g of human HGF cDNA) was injected into a cardiac infarction region. For a control group, an empty vector was introduced into a heart muscle having infarction. The human HGF concentration of heart tissue was measured with an enzyme linked immunosorbent assay (ELISA) using an anti-human HGF monoclonal antibody (Institute of Immunology, Tokyo, Japan) (Ueda H., Sawa Y., Matsumoto K. et al., Gene Transfection of Hepatocyte Growth Factor

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Attenuates reperfusion injury in the heart, Ann. Thorac. Surg., 1999, 67:1726-1731). The synthetic tissue produced in Example 30 was used. The cardiac infarction models produced by ligating LAD were subjected to three different therapies: 1) a cell sheet group; 2) a gene therapy group; 3) a combined therapy group; and 4) a control group. Changes in heart function and cardiomyocardial tissue were studied.

(Results)

For the synthetic tissue implanted group and the combined therapy group, the contractibility and expansibility of the heart were ameliorated. In addition, for the combined therapy group, it can be confirmed that vasculization was observed in the cardiac infarction portion, and the acceptance of implanted cells was improved.

(Conclusion)

By combining a synthetic tissue and a gene therapy, the decreased heart function ameliorating effect, the vasculization effect, and the cell protecting effect are obtained, so that a higher level of amelioration of the decreased heart function can be observed.

Although certain preferable embodiments have been described herein, it is not intended that such embodiments be construed as limitations on the scope of the invention except as set forth in the appended claims. Various other modifications and equivalents will be apparent to and can be readily made by those skilled in the art, after reading the description herein, without departing from the scope and spirit of this invention. All patents, published patent applications and publications cited herein are incorporated by reference as if set forth fully herein.

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INDUSTRIAL APPLICABILITY

5 The present invention usefully provides a basic
therapeutic method, technique, pharmaceutical agent, and
medical device for diseases which are conventionally
difficult to treat. Particularly, the present invention
provides an epoch-making therapy and prevention because it
promotes recovery to a substantially native state. The
10 present invention also provides a pharmaceutical agent, cell,
tissue, composition, system, kit, and the like, which are
used for such an epoch-making therapy and prevention.

15 There is a demand for repair and regeneration of joint
tissues, mainly including bones and cartilages which are
targeted by the present invention. The number of bone
fracture patients, which are targeted by bone regeneration,
accounts for several hundreds of thousands per year. It is
also said that there are 30 million potential patients having
20 osteoarthritis which is targeted by the cartilage
regenerative therapy. Thus, the potential market is huge.
The present invention is also highly useful for peripheral
industries. Acute competition has been started in the
regenerative medical research on joint tissues, mainly
25 including bone and cartilage. The synthetic tissue of the
present invention is a safe and original material made of
cells collected from an organism, such as a patient or the
like, and is highly useful in view of the lack of side effects
or the like.

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CLAIMS

1. An implantable synthetic tissue.
- 5 2. A synthetic tissue according to claim 1, which is biologically organized in the third dimensional direction.
3. A synthetic tissue according to claim 1, which has biological integration capability with surroundings.
- 10 4. A synthetic tissue according to claim 3, wherein the biological integration capability includes capability to adhere to surrounding cells and/or extracellular matrices.
- 15 5. A synthetic tissue according to claim 1, which comprises cells.
6. A synthetic tissue according to claim 1, which is substantially made of cells and a material derived from the
- 20 cells.
7. A synthetic tissue according to claim 1, which is substantially made of cells and an extracellular matrix (ECM) derived from the cells.
- 25 8. A synthetic tissue according to claim 7, wherein the extracellular matrix contains at least one selected from the group consisting of collagen I, collagen III, vitronectin and fibronectin.
- 30 9. A synthetic tissue according to claim 7, wherein the extracellular matrix contains collagen I, collagen III, vitronectin and fibronectin.

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10. A synthetic tissue according to claim 7, wherein the extracellular matrix contains vitronectin.
- 5 11. A synthetic tissue according to claim 7, wherein the extracellular matrix contains fibronectin.
12. A synthetic tissue according to claim 7, wherein the extracellular matrix contains collagen I and collagen III, the collagen constitutes 5% to 25% of the tissue, and the ratio of the collagen I to the collagen III is between 1:10 and 10:1.
- 10 13. A synthetic tissue according to claim 7, wherein the extracellular matrix and the cells are integrated together into a three-dimensional structure.
- 15 14. A synthetic tissue according to claim 7, wherein the extracellular matrix is diffusely distributed in the tissue.
- 20 15. A synthetic tissue according to claim 1, wherein an extracellular matrix is diffusely distributed, and the distribution densities of the extracellular matrix in two arbitrary sections of 1 cm² in the tissue have a ratio within a range of about 1:3 to about 3:1.
- 25 16. A synthetic tissue according to claim 1, which is heterologous, allogenic, isologous, or autogenous.
- 30 17. A synthetic tissue according to claim 1, which is free of scaffolds.
18. A synthetic tissue according to claim 1, which is used

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to implant cells.

19. A synthetic tissue according to claim 1, which is large sized.

5

20. A synthetic tissue according to claim 1, which has a volume of at least about 20 mm³.

10

21. A synthetic tissue according to claim 1, which is flexible.

22. A synthetic tissue according to claim 1, which is expandable and contractile.

15

23. A synthetic tissue according to claim 1, which can withstand heart pulsation.

20

24. A synthetic tissue according to claim 1, which is biologically organized in all three dimensional directions.

25. A synthetic tissue according to claim 24, wherein the biological integration is selected from the group consisting of internal binding of extracellular matrix, electrical integration, and intercellular signal transduction.

25

26. A synthetic tissue according to claim 1, which has a tissue strength which allows the synthetic tissue to be clinically applicable.

30

27. A synthetic tissue according to claim 26, wherein the strength is a break strength of about 0.02 N to about 2 N.

28. A synthetic tissue according to claim 26, wherein the

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tissue strength is sufficient to provide self-supporting ability.

5 29. A synthetic tissue according to claim 28, wherein the self-supporting ability is characterized in that the synthetic tissue is not substantially broken when the synthetic tissue is picked up using forceps having a tip area of 0.05 to 3.0 mm².

10 30. A synthetic tissue according to claim 28, wherein the self-supporting ability is characterized in that the synthetic tissue is not broken when the synthetic tissue is picked up with a hand.

15 31. A synthetic tissue according to claim 26, wherein the site to which the synthetic tissue is intended to be applied, includes a heart.

20 32. A synthetic tissue according to claim 26, wherein the site to which the synthetic tissue is intended to be applied, includes an intervertebral disk, a meniscus, a cartilage, a bone, a ligament, or a tendon.

25 33. A synthetic tissue according to claim 26, wherein:
the synthetic tissue is a cartilage, an intervertebral disk, a meniscus, a ligament, or a tendon; and

30 the synthetic tissue remains attached without an additional fixation procedure, after the synthetic tissue is implanted into an injured portion of the intra-articular tissue.

34. A method for producing a synthetic tissue, comprising

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the steps of:

A) providing cells;

5 B) placing the cells in a container, the container having cell culture medium containing an ECM synthesis promoting agent and having a sufficient base area which can accommodate a synthetic tissue having a desired size;

10 C) culturing the cells in the container along with the cell culture medium containing the ECM synthesis promoting agent for a period of time sufficient for formation of the synthetic tissue having the desired size; and

D) detaching the cells from the container.

15 35. A method according to claim 34, wherein a stimulus for inducing tissue contraction is applied in the detaching step.

36. A method according to claim 35, wherein the stimulus includes a physical or chemical stimulus.

20 37. A method according to claim 36, wherein the physical stimulus includes shaking of the container, pipetting, or deformation of the container.

25 38. A method according to claim 34, wherein the detaching step includes adding an actin regulatory agent.

30 39. A method according to claim 38, wherein the actin regulatory agent includes a chemical substance selected from the group consisting of actin depolymerizing agents and actin polymerizing agents.

40. A method according to claim 39, wherein the actin depolymerizing agent is selected from the group consisting of Slingshot, cofilin, cyclase associated protein (CAP),

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actin interacting protein 1 (AIP1), actin depolymerizing factor (ADF), destrin, depactin, actophorin, cytochalasin, and NGF (nerve growth factor).

5 41. A method according to claim 39, wherein the actin polymerizing agent is selected from the group consisting of RhoA, mDi, profilin, Rac1, IRSp53, WAVE2, ROCK, LIM kinase, cofilin, cdc42, N-WASP, Arp2/3, Drf3, Mena, lysophosphatidic acid (LPA), insulin, platelet derived growth factor (PDGF)
10 a, PDGFb, chemokine, and transforming growth factor (TGF) β .

42. A method according to claim 34, wherein the container
15 is free of scaffolds.

43. A method according to claim 34, wherein the cells are
first cultured in monolayer culture.

44. A method according to claim 34, wherein the ECM synthesis
20 promoting agent includes TGF β 1, TGF β 3, ascorbic acid, ascorbic acid 2-phosphate, or a derivative or salt thereof.

45. A method according to claim 44, wherein the ascorbic
25 acid, ascorbic acid 2-phosphate, or the derivative or salt thereof is present at a concentration of at least 0.1 mM.

46. A method according to claim 44, wherein the TGF β 1 or
TGF β 3 is present at a concentration of at least 1 ng/ml.

30 47. A method according to claim 34, wherein the cells are placed at a concentration of 5×10^4 to 5×10^5 cells per 1 cm^2 , and the ECM synthesis promoting agent is ascorbic acid, ascorbic acid 2-phosphate, or a derivative or salt thereof,

- 186 -

and the ascorbic acid, ascorbic acid 2-phosphate, or the derivative or salt thereof is provided at a concentration of at least 0.1 mM.

5 48. A method according to claim 34, further comprising causing the synthetic tissue to detach from the container and self-contract.

10 49. A method according to claim 48, wherein the detaching and self-contraction are achieved by providing a physical stimulus to the container.

15 50. A method according to claim 48, wherein the detachment and self-contraction are achieved by providing a chemical stimulus to the container.

51. A method according to claim 34, wherein the sufficient period of time is at least 3 days.

20 52. A method according to claim 34, wherein the sufficient period of time is at least 3 days and a period of time required for the synthetic tissue to be spontaneously detached from the container at a maximum.

25 53. A method according to claim 52, wherein the period of time required for the synthetic tissue to be spontaneously detached from the container is at least 40 days.

30 54. A method according to claim 34, further comprising:
causing the synthetic tissue to differentiate.

55. A method according to claim 54, wherein the differentiation includes osteogenesis, chondrogenesis,

- 187 -

adipogenesis, tendon differentiation, and ligament differentiation.

5 56. A method according to claim 55, wherein the osteogenesis is performed in medium containing dexamethasone, β -glycerophosphate, and ascorbic acid 2-phosphate.

10 57. A method according to claim 56, wherein the medium contains at least one selected from the group consisting of BMP (bone morphogenetic protein)-2, BMP-4, and BMP-7.

15 58. A method according to claim 55, wherein the chondrogenesis is performed in medium containing pyruvic acid, dexamethasone, ascorbic acid 2-phosphate, insulin, transferrin, and selenious acid.

20 59. A method according to claim 58, wherein the medium contains at least one selected from the group consisting of BMP-2, BMP-4, BMP-7, TGF(transforming growth factor)- β 1 and TGF- β 3.

25 60. A method according to claim 54, wherein the differentiation step is performed before or after the detaching step.

61. A method according to claim 54, wherein the differentiation step is performed after the detaching step.

30 62. A method according to claim 34, wherein the cell includes cells of 3 or more passages.

63. A method according to claim 34, wherein the cells include cells of 3 to 8 passages.

- 188 -

64. A method according to claim 34, wherein the cells are provided at a cell density of 5.0×10^4 to 5.0×10^6 cells/cm².

5 65. A method according to claim 34, wherein the cells include myoblasts.

66. A method according to claim 34, wherein the cells include fat-derived cells.

10

67. A method according to claim 34, wherein the cells include synovium-derived cells.

15 68. A method according to claim 34, wherein the cells include mesenchymal stem cells.

69. A method according to claim 68, wherein the mesenchymal stem cells are derived from an adipose tissue, a synovial membrane, a tendon, a bone, or a bone marrow.

20

70. A method according to claim 34, further comprising:
producing a plurality of the synthetic tissues and
attaching the plurality of the synthetic tissues together
to be integrated.

25

71. A cell culture composition for producing a synthetic tissue from cells, comprising:

A) an element for maintaining the cells; and

B) an extracellular matrix synthesis promoting

30 agent.

72. A method according to claim 68, wherein the ECM synthesis promoting agent includes TGF β 1, TGF β 3, ascorbic acid,

- 189 -

ascorbic acid 2-phosphate, or a derivative or salt thereof.

73. A method according to claim 72, wherein TGF β 1 or TGF β 3 is present at a concentration of at least 1 ng/ml, or ascorbic acid, ascorbic acid 2-phosphate, or the derivative or salt thereof is present at a concentration of at least 0.1 mM.

74. A complex for reinforcing a portion of an organism, comprising cells and a component derived from the cells.

75. A complex according to claim 74, which has biological integration capability with surroundings.

76. A complex according to claim 75, wherein the biological integration capability include capability to adhere to surrounding cells and/or extracellular matrices.

77. A complex according to claim 74, which is substantially made of cells and a material derived from the cells.

78. A complex according to claim 74, which is substantially made of cells and an extracellular matrix derived from the cells.

79. A synthetic tissue according to claim 78, wherein the extracellular matrix is selected from the group consisting of collagen I, collagen III, vitronectin and fibronectin.

80. A complex according to claim 78, wherein the extracellular matrix and the cells are integrated together into a three-dimensional structure.

81. A complex according to claim 78, wherein the

- 190 -

extracellular matrix is provided on a surface of the complex.

82. A complex according to claim 78, wherein the
extracellular matrix is diffusedly distributed on a surface
5 of the complex.

83. A complex according to claim 74, wherein an extracellular
matrix is diffusedly distributed on a surface of the complex,
and the distribution densities of the extracellular matrix
10 in two arbitrary sections of 1 cm² in the complex have a ratio
within a range of about 1:3 to about 3:1.

84. A complex according to claim 78, wherein the
extracellular matrix includes fibronectin or vitronectin.
15

85. A complex according to claim 74, which is heterologous,
allogenic, isologous, or autogenous.

86. A complex according to claim 74, wherein the portion
20 includes a bag-shaped organ.

87. A complex according to claim 86, wherein the bag-shaped
organ includes a heart.

88. A complex according to claim 74, wherein the portion
25 includes a bone or cartilage tissue.

89. A complex according to claim 74, wherein the portion
includes avascular tissue.
30

90. A complex according to claim 74, wherein the portion
includes an intervertebral disk, a meniscus, a ligament,
or a tendon.

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- 5 91. A complex according to claim 74, wherein the reinforcement is achieved by replacing the portion with the complex or providing the complex to cover the portion, or both.
92. A complex according to claim 74, which resists the expansion and contraction of the portion.
- 10 93. A complex according to claim 74, which has biological integration.
- 15 94. A complex according to claim 74, wherein the biological integration selected from the group consisting of internal binding of extracellular matrix, electrical integration, and intercellular signal transduction.
- 20 95. A complex according to claim 74, which is formed by culturing cells in the presence of an ECM synthesis promoting agent.
96. A complex according to claim 74, which has self-supporting ability.
- 25 97. A method for reinforcing a portion of an organism, comprising the steps of:
- 30 A) replacing the portion with a complex comprising cells and a component derived from the cells or providing the complex to cover the portion, or both; and
- B) holding the complex for a sufficient period of time for biologically adhering the complex to the portion.
98. A method according to claim 97, wherein the adhesion

- 192 -

is achieved by adhesion between extracellular matrix and extracellular matrix.

5 99. A method according to claim 97, which has biological integration capability with surroundings.

10 100. A method according to claim 99, wherein the biological integration capability include capability to adhere to surrounding cells and/or extracellular matrices.

101. A method according to claim 97, which is substantially made of cells and a material derived from the cells.

15 102. A method according to claim 97, which is substantially made of cells and an extracellular matrix derived from the cells.

20 103. A method according to claim 102, wherein the extracellular matrix contains one selected from the group consisting of collagen I, collagen III, vitronectin and fibronectin.

25 104. A method according to claim 102, wherein the extracellular matrix contains all of collagen I, collagen III, vitronectin and fibronectin.

105. A method according to claim 102, wherein the extracellular matrix contains vitronectin.

30 106. A method according to claim 102, wherein the extracellular matrix contains fibronectin.

107. A method according to claim 97, wherein an extracellular

- 193 -

matrix is provided on a surface of the complex.

108. A method according to claim 97, wherein an extracellular matrix is diffusedly distributed on a surface of the complex.

5

109. A method according to claim 97, wherein an extracellular matrix is diffusedly distributed on a surface of the complex, and the distribution densities of the extracellular matrix in two arbitrary sections of 1 cm² have a ratio within a range of about 1:3 to about 3:1.

10

110. A complex according to claim 97, wherein an extracellular matrix is diffusedly distributed on a surface of the complex, and the distribution densities of the extracellular matrix in two arbitrary sections of 1 cm² have a ratio within a range of about 1:2 to about 2:1.

15

111. A method according to claim 97, which is heterologous, allogenic, isologous, or autogenous.

20

112. A method according to claim 97, wherein the portion includes a bag-shaped organ.

113. A method according to claim 112, wherein the bag-shaped organ includes a heart.

25

114. A method according to claim 97, wherein the complex resists the expansion and contraction of the portion.

115. A method according to claim 97, wherein the complex has biological integration.

30

116. A method according to claim 115, wherein the biological

- 194 -

integration selected from the group consisting of internal binding of extracellular matrix, electrical integration, and intercellular signal transduction.

5 117. A method according to claim 97, further comprising:
forming the complex by culturing the cells in the presence of an ECM synthesis promoting agent.

10 118. A method according to claim 97, wherein the portion is a heart and the heart has a disease or disorder selected from the group consisting of heart failure, ischemic heart disease, myocardial infarct, cardiomyopathy, myocarditis, hypertrophic cardiomyopathy, dilated phase hypertrophic cardiomyopathy, and dilated cardiomyopathy.

15 119. A method according to claim 97, wherein the portion includes an avascular lesion.

20 120. A method according to claim 97, wherein the portion includes a vascular lesion.

121. A method according to claim 97, wherein the portion includes a bone or a cartilage.

25 122. A method according to claim 97, wherein the portion includes an intervertebral disk, a meniscus, a ligament, or a tendon.

30 123. A method according to claim 97, wherein the portion includes a bone or a cartilage, and the bone or the cartilage is damaged or degenerated.

124. A method according to claim 97, wherein the portion

- 195 -

includes intractable fracture, osteonecrosis, cartilage injury, meniscus injury, ligament injury, tendon injury, cartilage degeneration, meniscus degeneration, intervertebral disk denaturation, ligament degeneration, or tendon degeneration.

125. A method according to claim 97, wherein the sufficient period of time is at least 10 days.
126. A method according to claim 97, wherein the complex has self-supporting ability.
127. A method according to claim 97, which has biological integration capability with surroundings.
128. A method according to claim 97, which is substantially made of cells and an extracellular matrix derived from the cells.
129. A method according to claim 97, further comprising implanting another synthetic tissue.
130. A method according to claim 129, wherein the other synthetic tissue is an artificial bone or a microfibrinous collagen medical device.
131. A method according to claim 97, which is substantially made of cells and an extracellular matrix derived from the cells, wherein the other synthetic tissue is an artificial bone or a microfibrinous collagen medical device.
132. A method according to claim 130, the artificial bone includes hydroxyapatite.

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133. A method for treating a portion of an organism, comprising the steps of:

5 A) replacing the portion with a complex comprising cells and a component derived from the cells or providing the complex to cover the portion, or both; and

 B) holding the complex for a sufficient period of time for restoring a condition of the portion.

10 134. A method according to claim 133, wherein the treatment is for the treatment, prevention, or reinforcement of a disease, disorder, or condition of a heart, a bone, a cartilage, a ligament, a tendon, a meniscus, or an intervertebral disk.

15 135. A method according to claim 133, wherein the complex has self-supporting ability.

20 136. A method according to claim 133, wherein the complex has biological integration capability with surroundings.

 137. A method according to claim 133, wherein the complex is substantially made of cells and an extracellular matrix derived from the cells.

25 138. A method according to claim 133, further comprising implanting another synthetic tissue in addition to the replacement or coverage of the portion.

30 139. A method according to claim 138, wherein the other synthetic tissue includes an artificial bone or a microfibrinous collagen medical device.

140. A method according to claim 133, which is substantially made of cells and an extracellular matrix derived from the cells, wherein the other synthetic tissue includes an artificial bone or a microfibrinous collagen medical device.

5

141. A method according to claim 139, the artificial bone includes hydroxyapatite.

142. A method for producing a synthetic tissue, comprising the steps of:

10

A) providing cells;

B) placing the cells in a container, the container having cell culture medium containing an ECM synthesis promoting agent and having a sufficient base area which can accommodate a synthetic tissue having a desired size;

15

C) culturing the cells in the container along with the cell culture medium containing the ECM synthesis promoting agent for a period of time sufficient for formation of the synthetic tissue having the desired size; and

20

D) regulating a thickness of the synthetic tissue by a physical or chemical stimulus to a desired thickness.

143. A method according to claim 142, wherein the physical stimulus includes shear stress between the synthetic tissue and the container, deformation of the base of the container, shaking of the container, or pipetting.

25

144. A method according to claim 142, wherein the chemical stimulus is obtained by using a chemical substance selected from the group consisting of actin depolymerizing agents and actin polymerizing agents.

30

145. A method according to claim 144, wherein the actin

- 198 -

depolymerizing agent is selected from the group consisting of Slingshot, cofilin, CAP (cyclase associated protein), AIP1 (actin interacting protein 1), ADF (actin depolymerizing factor), destrin, depactin, actophorin, cytochalasin, and NGF (nerve growth factor).

146. A method according to claim 144, wherein the actin polymerizing agent is selected from the group consisting of RhoA, mDi, profilin, Rac1, IRSp53, WAVE2, ROCK, LIM kinase, cofilin, cdc42, N-WASP, Arp2/3, Drf3, Mena, LPA (lysophosphatidic acid), insulin, PDGF (platelet derived growth factor), PDGFb, chemokine, and TGF (transforming growth factor) β .

147. A method according to claim 144, wherein the desired thickness is regulated by adjusting a ratio of the actin depolymerizing agent to the actin polymerizing agent.

148. A method according to claim 142, further comprising:
producing a plurality of the synthetic tissues and attaching the plurality of the synthetic tissues together to be integrated.

149. A tissue complex, comprising an implantable synthetic tissue and another synthetic tissue.

150. A tissue complex according to claim 149, wherein the implantable synthetic tissue is substantially made of cells and a material derived from the cells.

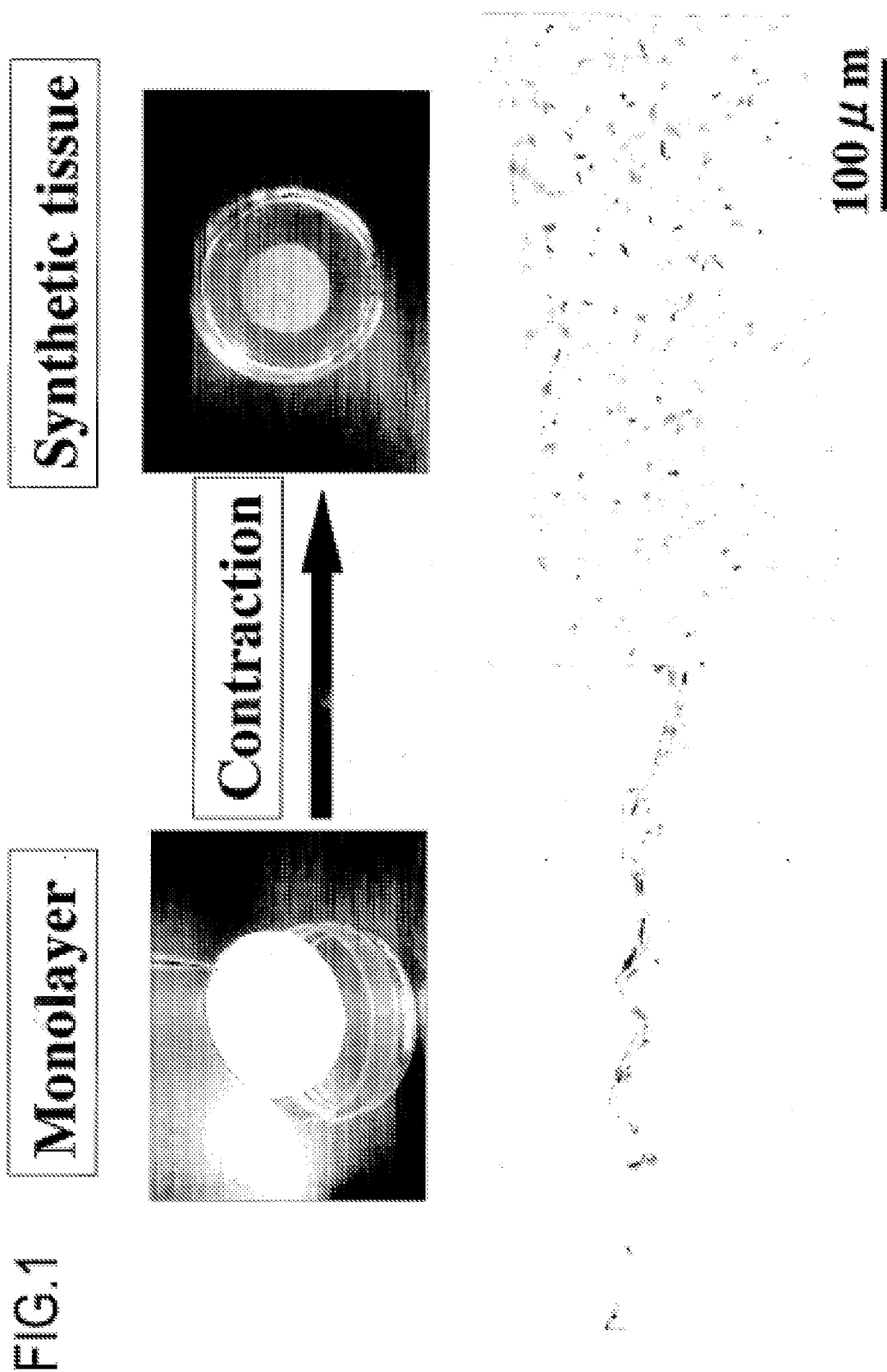
151. A tissue complex according to claim 149, wherein the implantable synthetic tissue is substantially made of cells and an extracellular matrix derived from the cells.

152. A tissue complex according to claim 151, wherein the extracellular matrix is selected from the group consisting of collagen I, collagen III, vitronectin, and fibronectin.
- 5 153. A tissue complex according to claim 151, wherein the extracellular matrix contains all of collagen I, collagen III, vitronectin, and fibronectin.
- 10 154. A tissue complex according to claim 149, wherein the other synthetic tissue includes an artificial bone or a microfibrinous collagen medical device.
- 15 155. A tissue complex according to claim 154, the artificial bone includes hydroxyapatite.
156. A tissue complex according to claim 149, the implantable synthetic tissue is biologically integrated with the other synthetic tissue.
- 20 157. A tissue complex according to claim 156, wherein the biological integration is achieved via an extracellular matrix.
- 25 158. A composition for use in producing a synthetic tissue having a desired thickness, comprising a chemical substance selected from the group consisting of actin depolymerizing agents and actin polymerizing agents.
- 30 159. A composition according to claim 158, wherein the actin depolymerizing agent is selected from the group consisting of Slingshot, cofilin, CAP (cyclase associated protein), AIP1 (actin interacting protein 1), ADF (actin depolymerizing

- 200 -

factor), destrin, depactin, actophorin, cytochalasin, and NGF (nerve growth factor).

150. A composition according to claim 158, wherein the actin
5 polymerizing agent is selected from the group consisting
of RhoA, mDi, profilin, Rac1, IRSp53, WAVE2, ROCK, LIM kinase,
cofilin, cdc42, N-WASP, Arp2/3, Drf3, Mena, LPA
(lysophosphatidic acid), insulin, PDGF (platelet derived
growth factor) α , PDGFB, chemokine, and TGF (transforming
10 growth factor) β .



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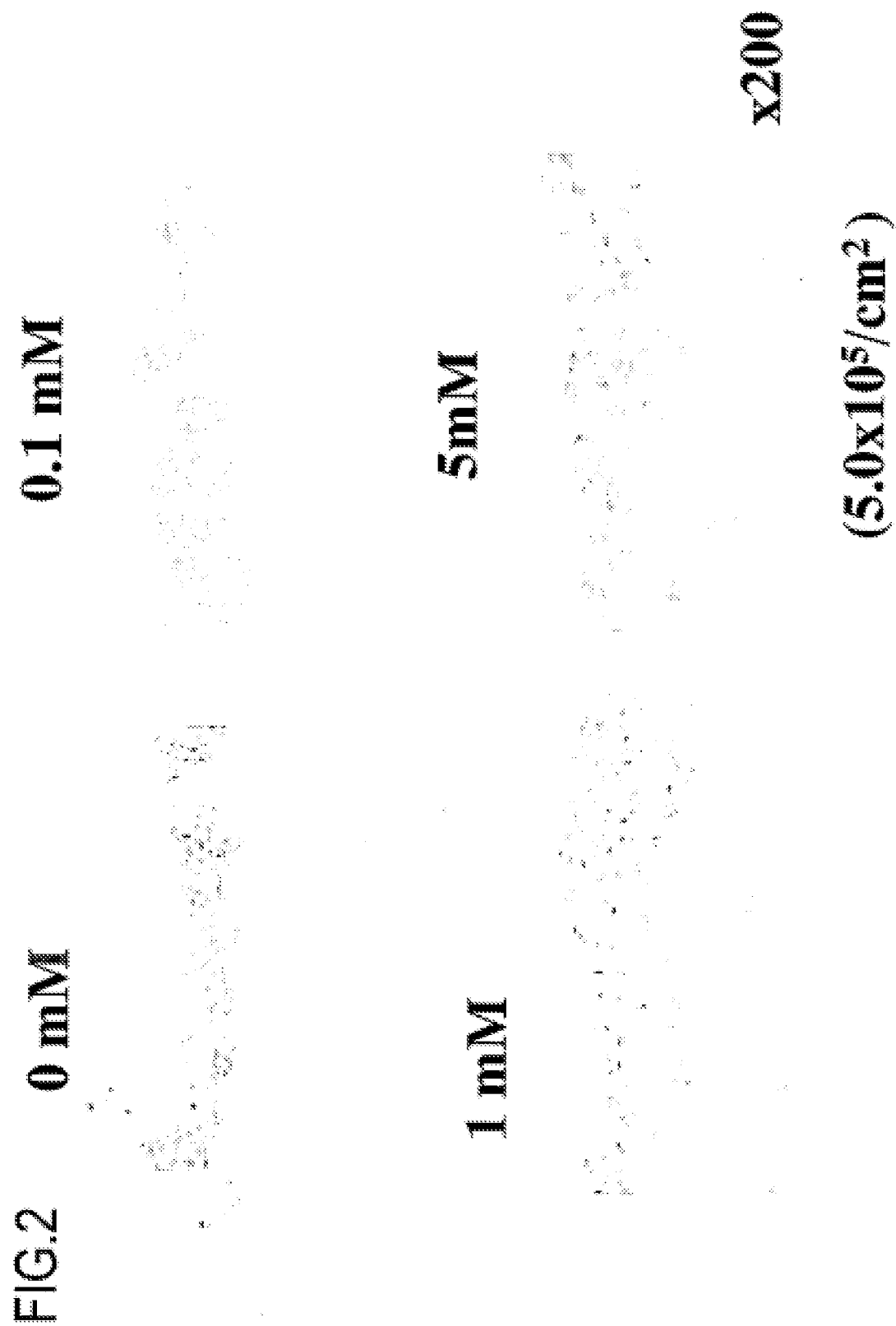


FIG.3

Day 3

Day 7

Day 14

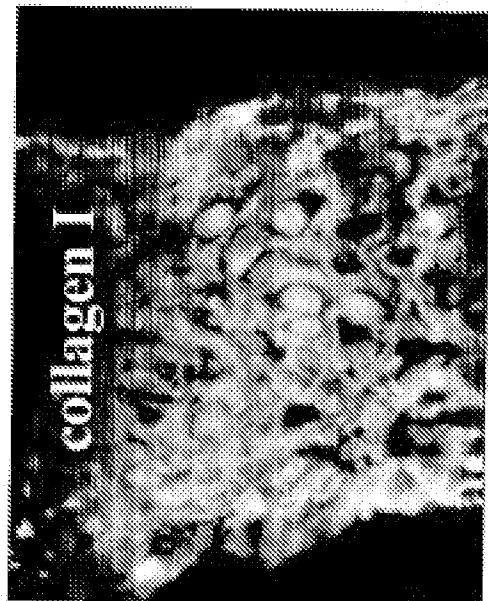
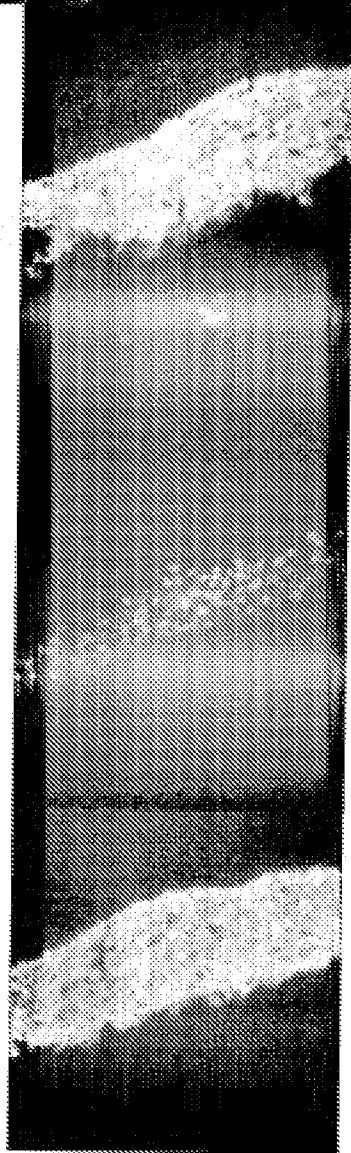
Day 21

Day 1. It is difficult to detach cell sheet

(1×10^6 cell/ cm^2 Asc-2P 1mM)

FIG.4

collagen I collagen II collagen III



fibronectins vitronectin negative control

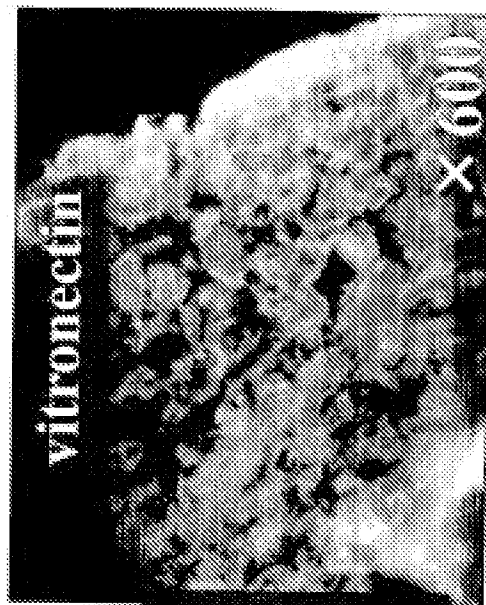
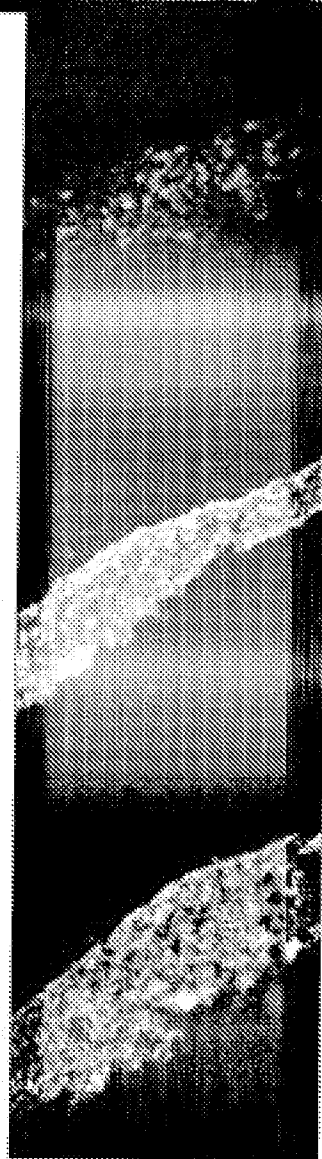


FIG.5

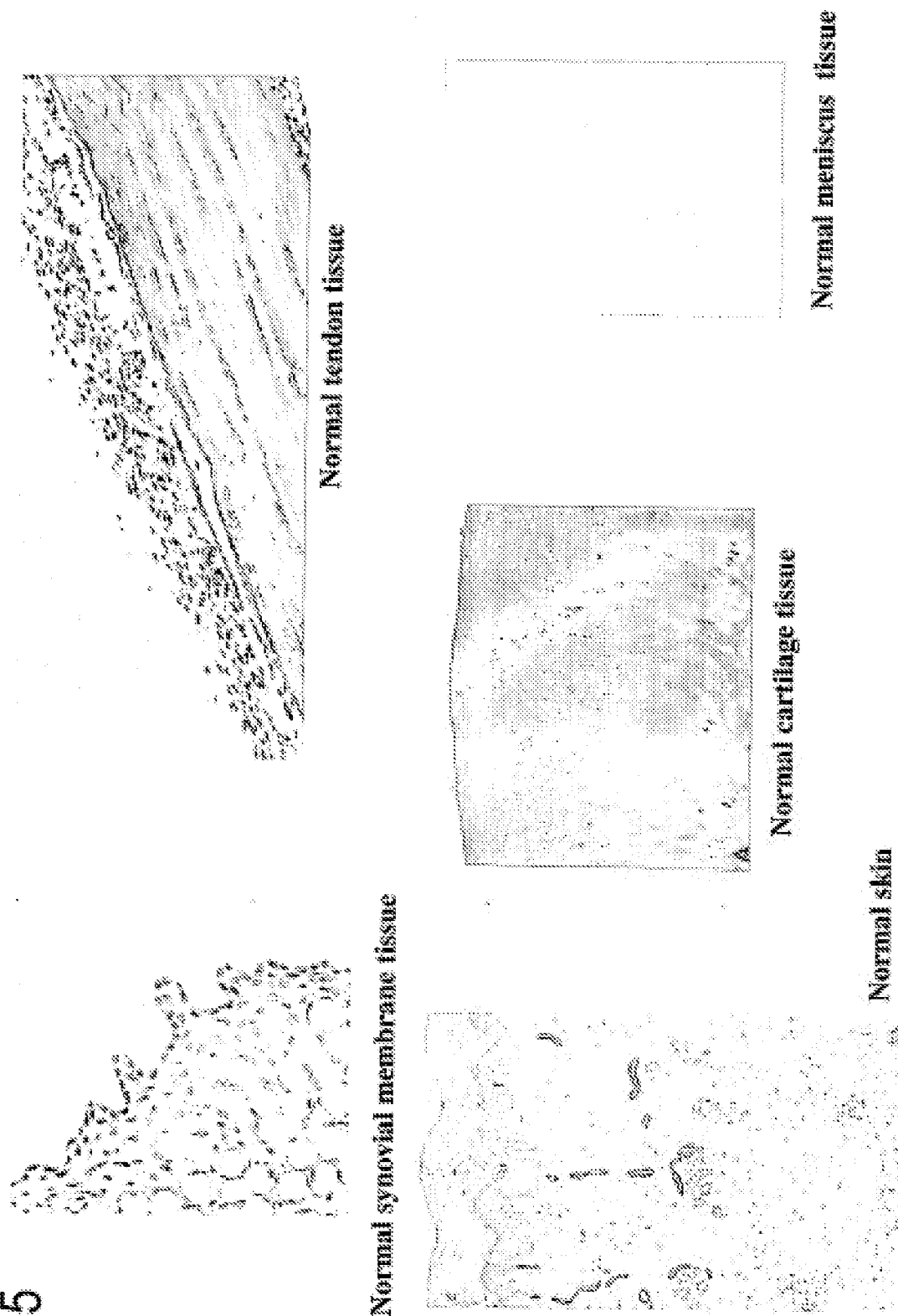
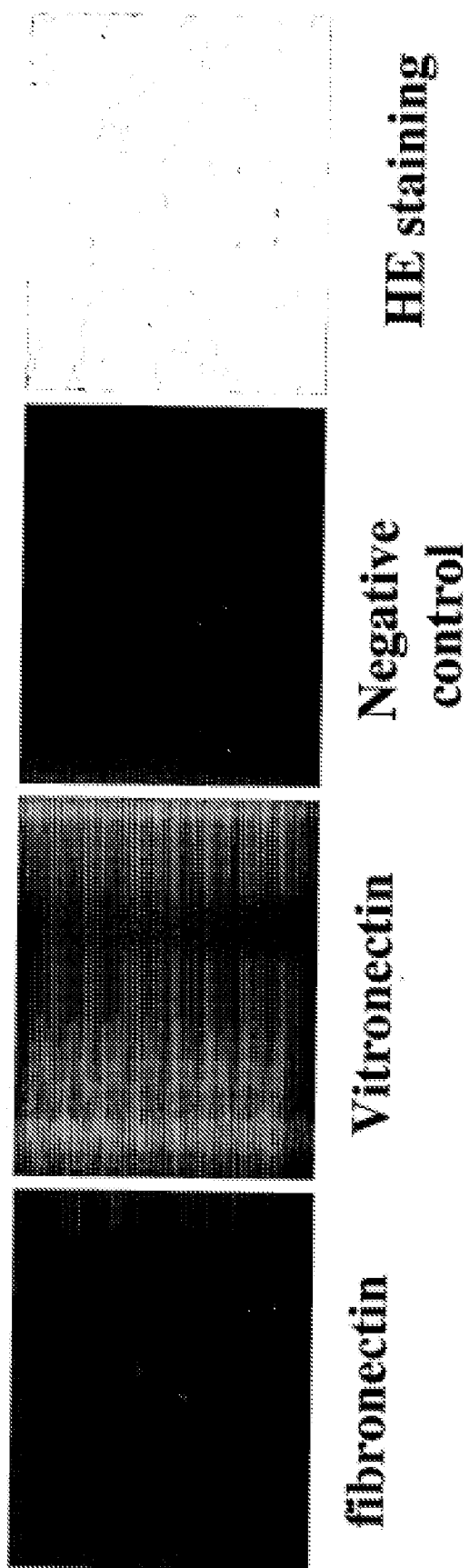
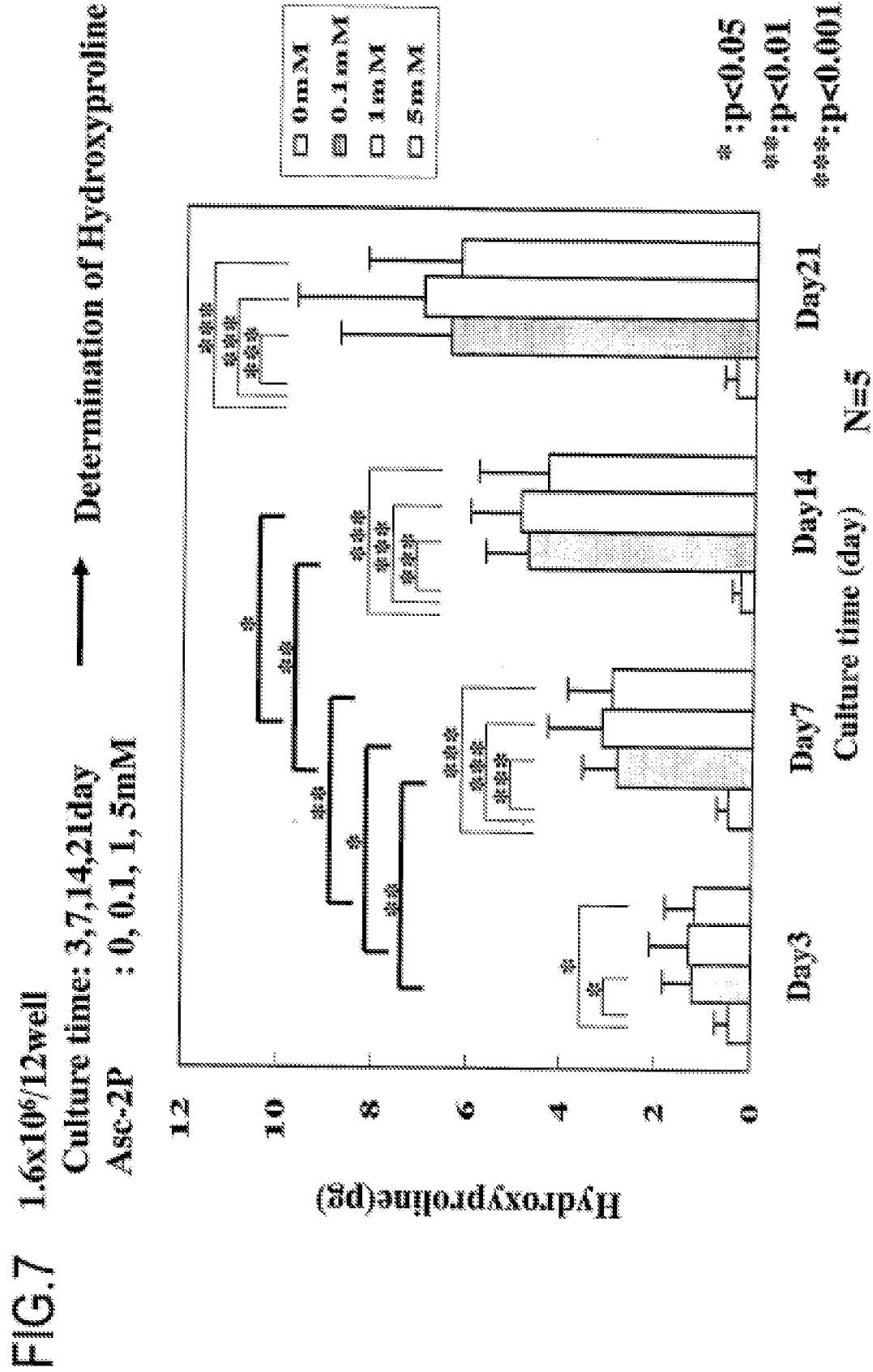


FIG.6





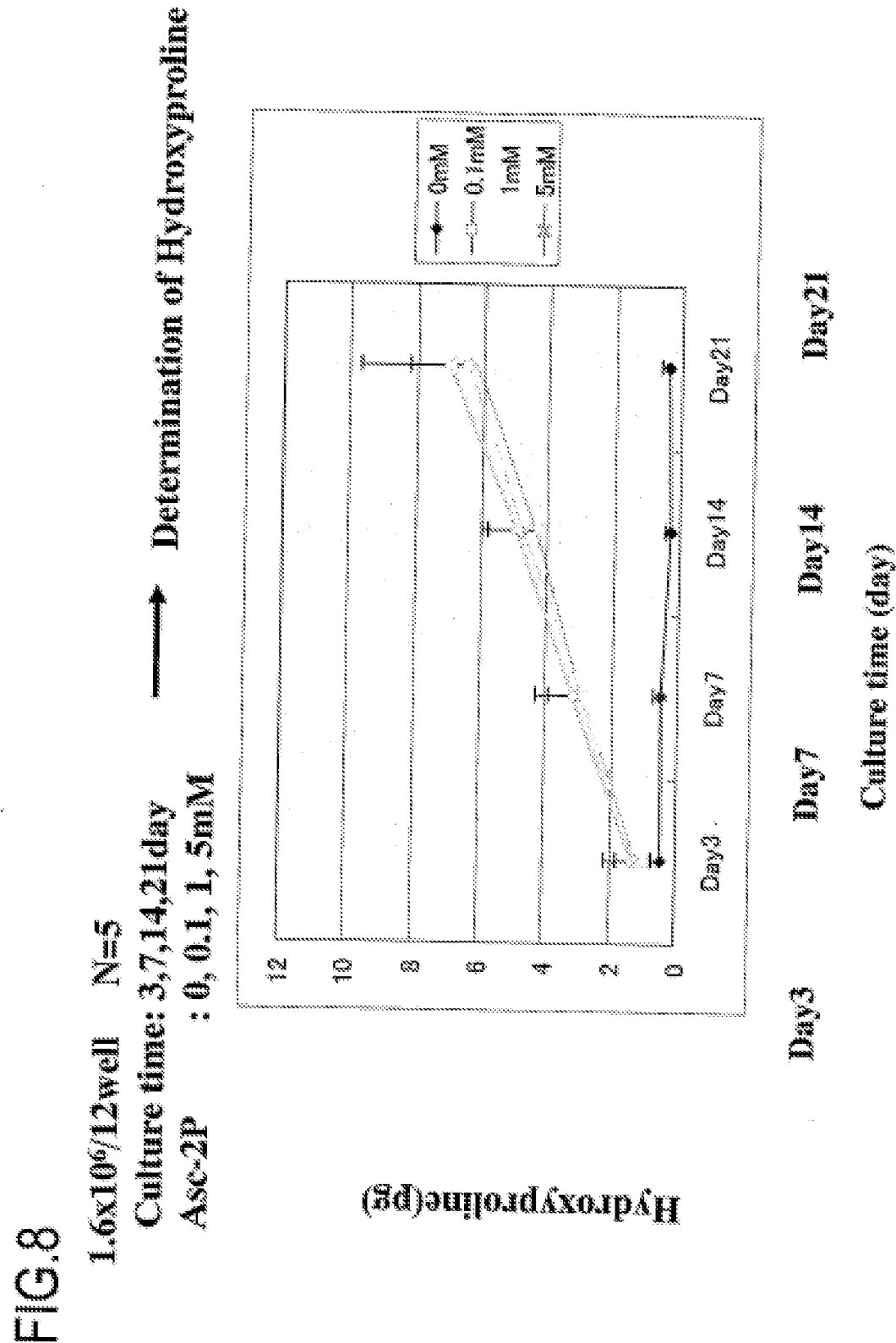


FIG.9

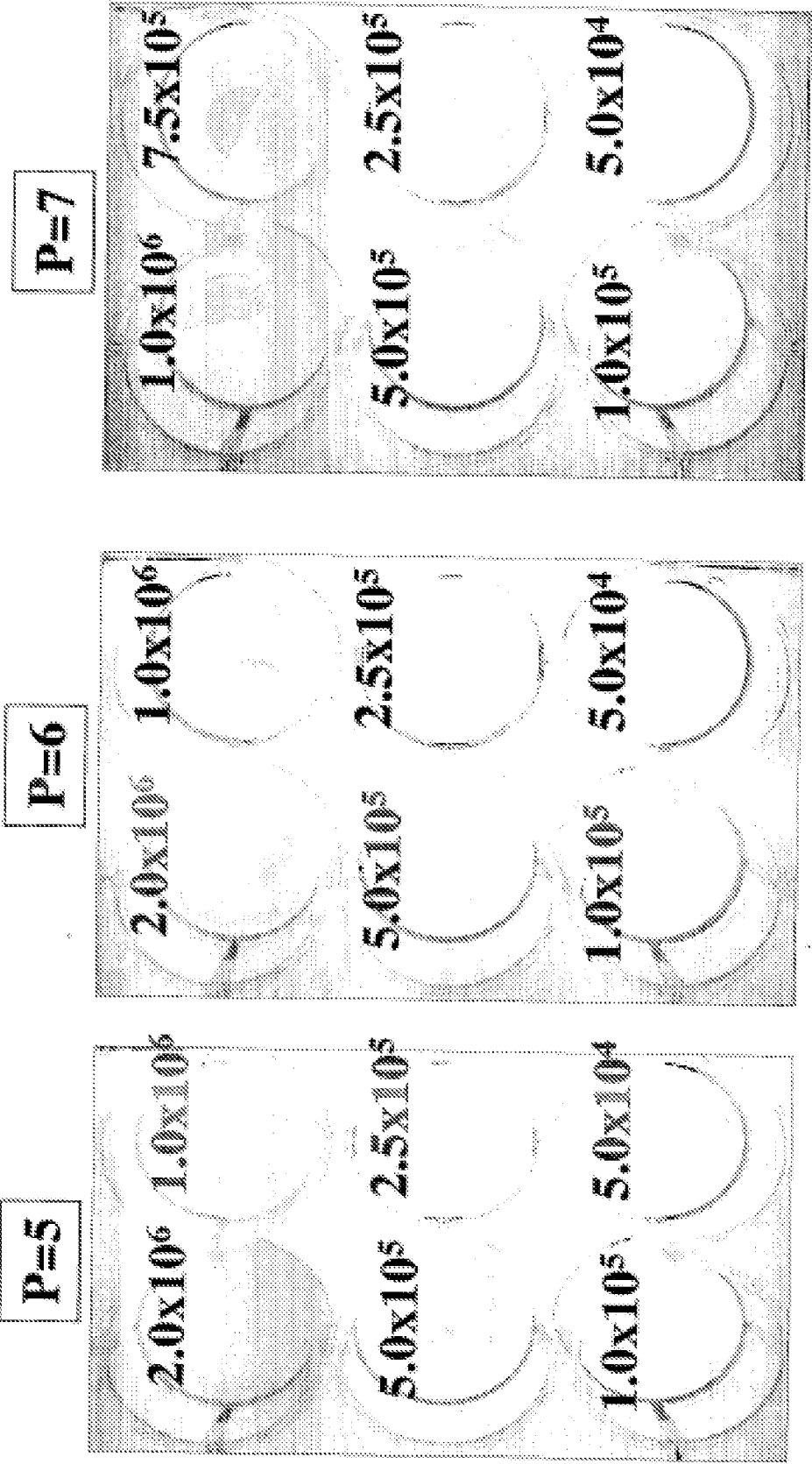


FIG.10

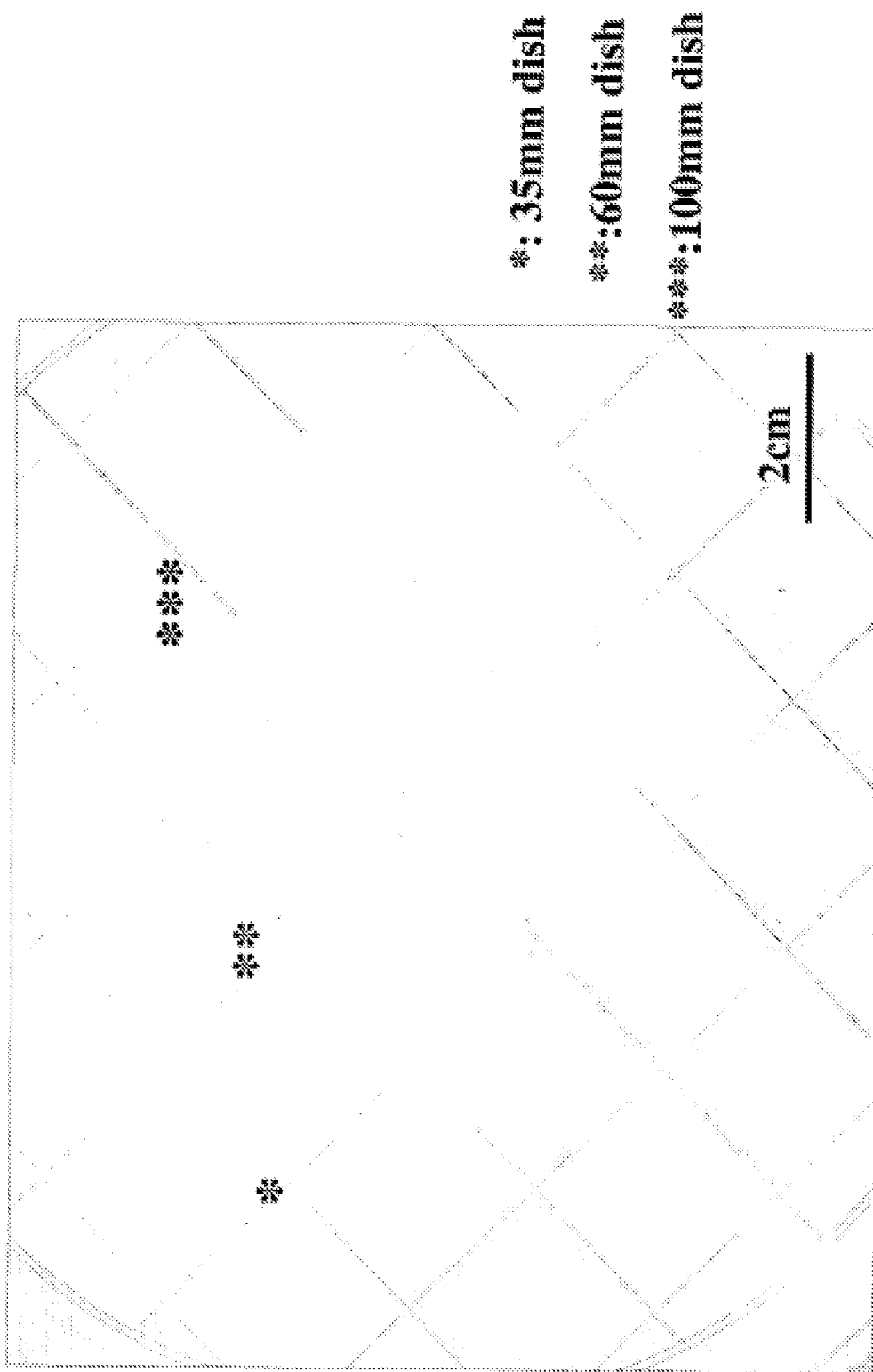


FIG.11

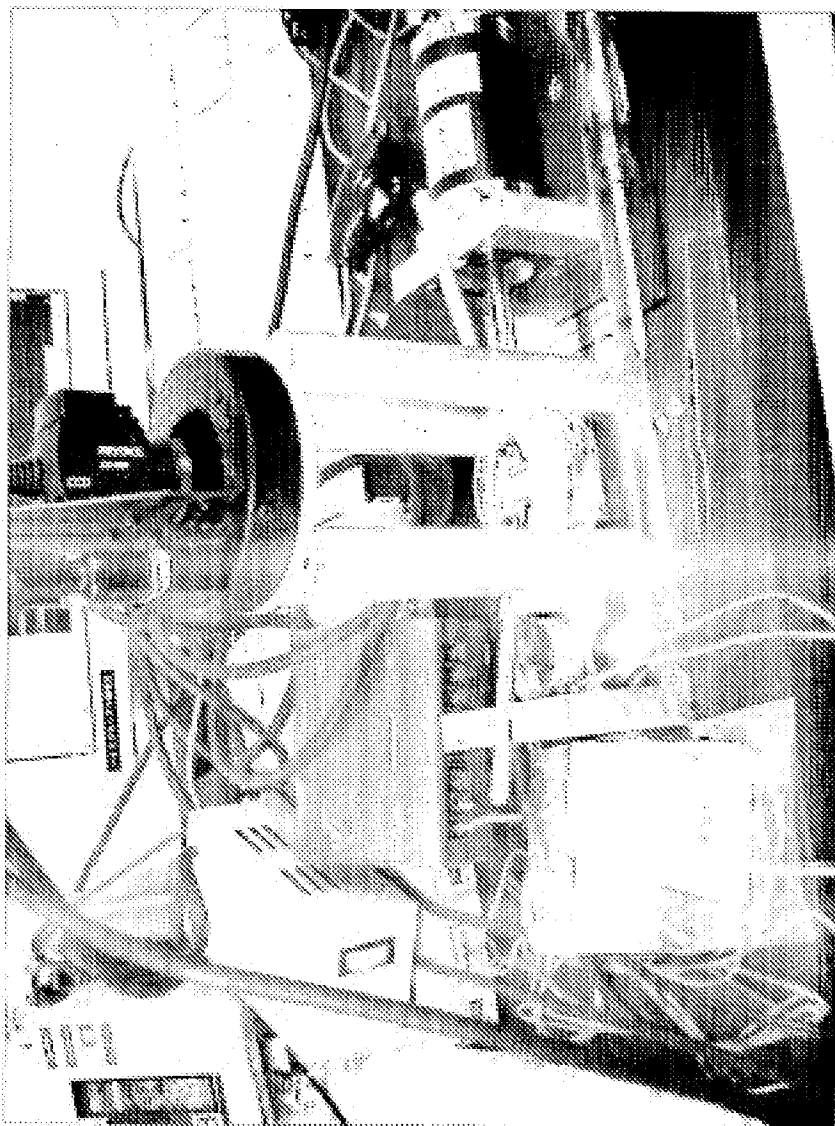


FIG.12

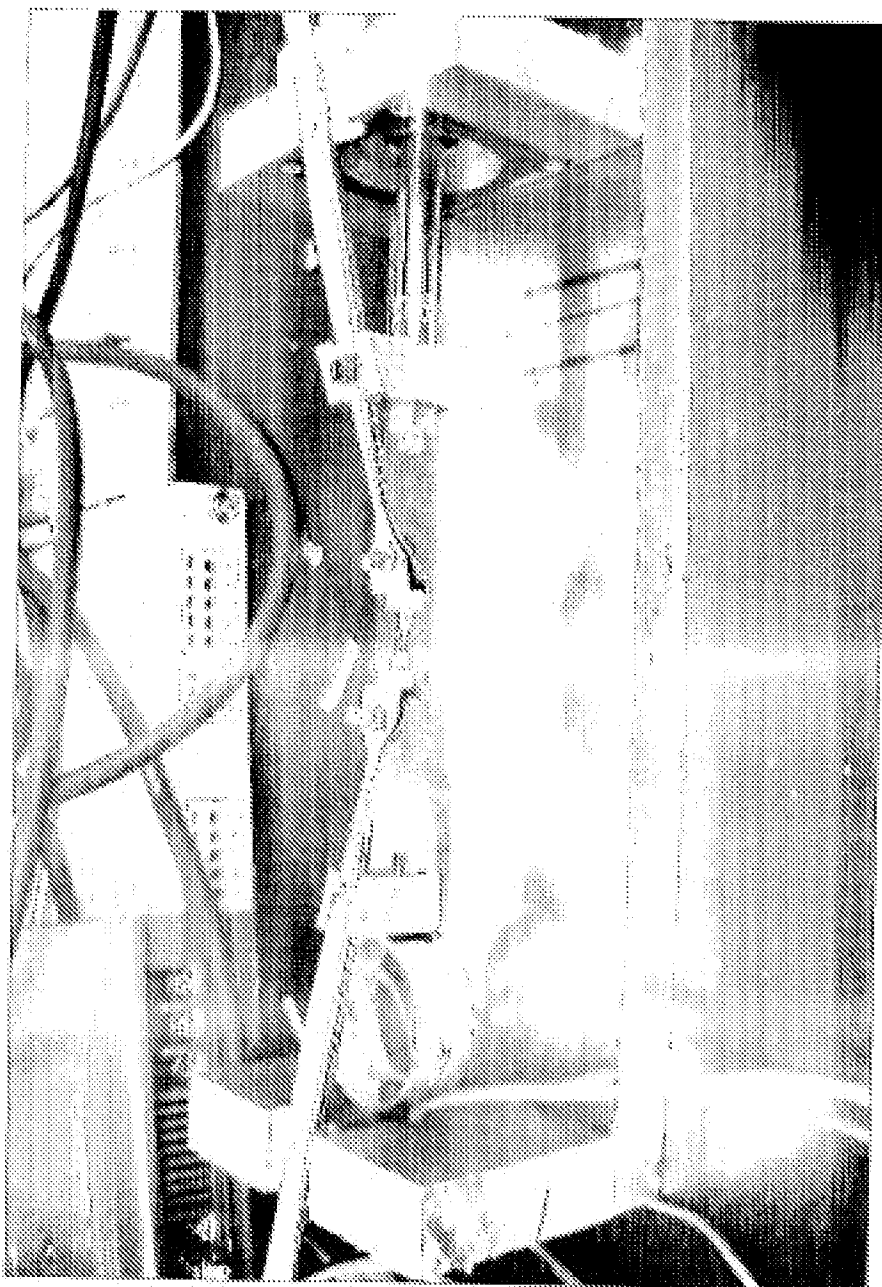
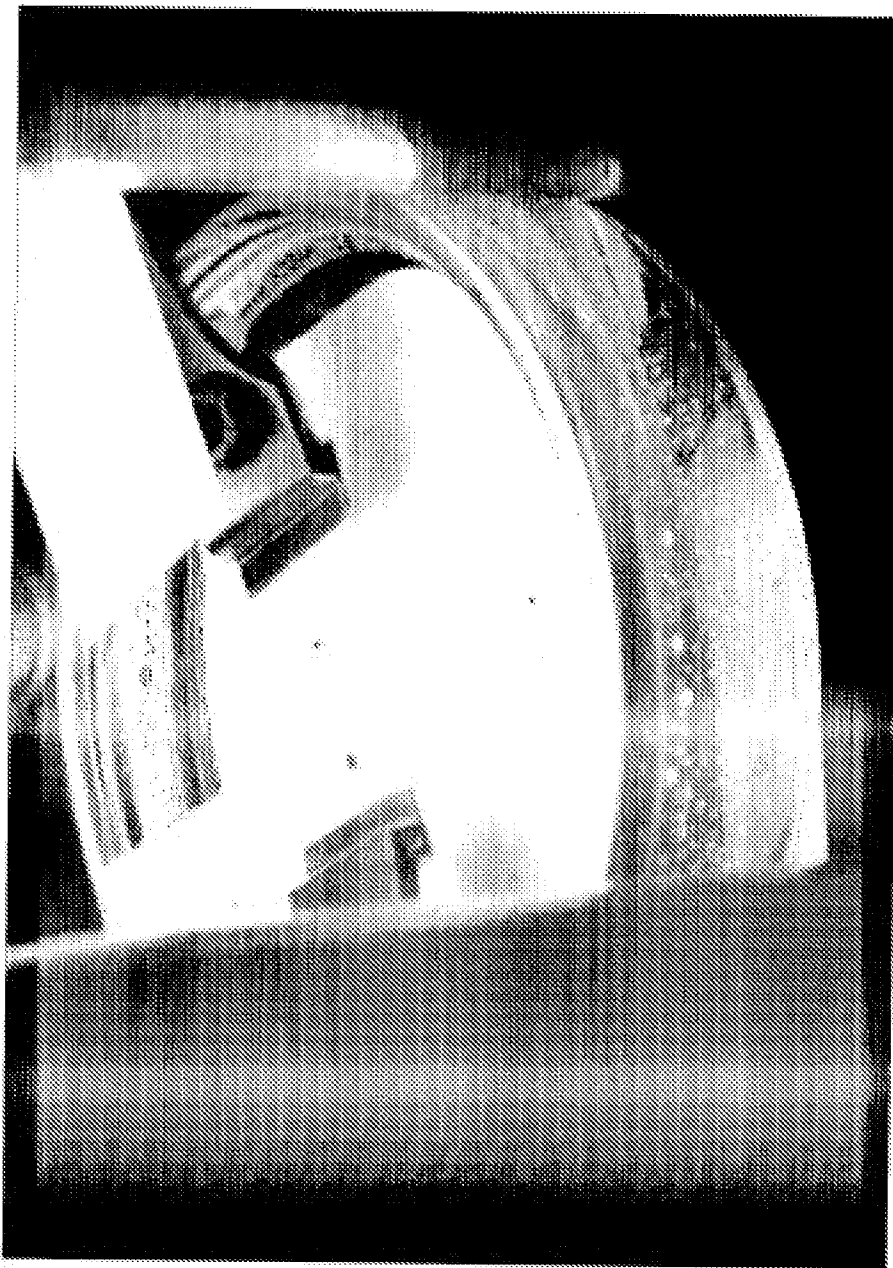


FIG.13



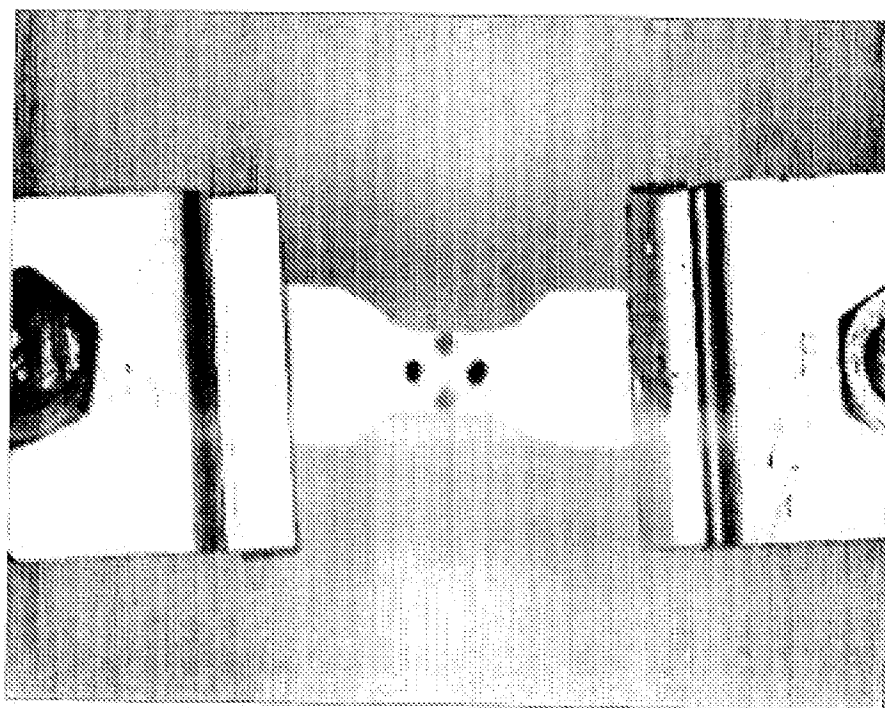


FIG.14

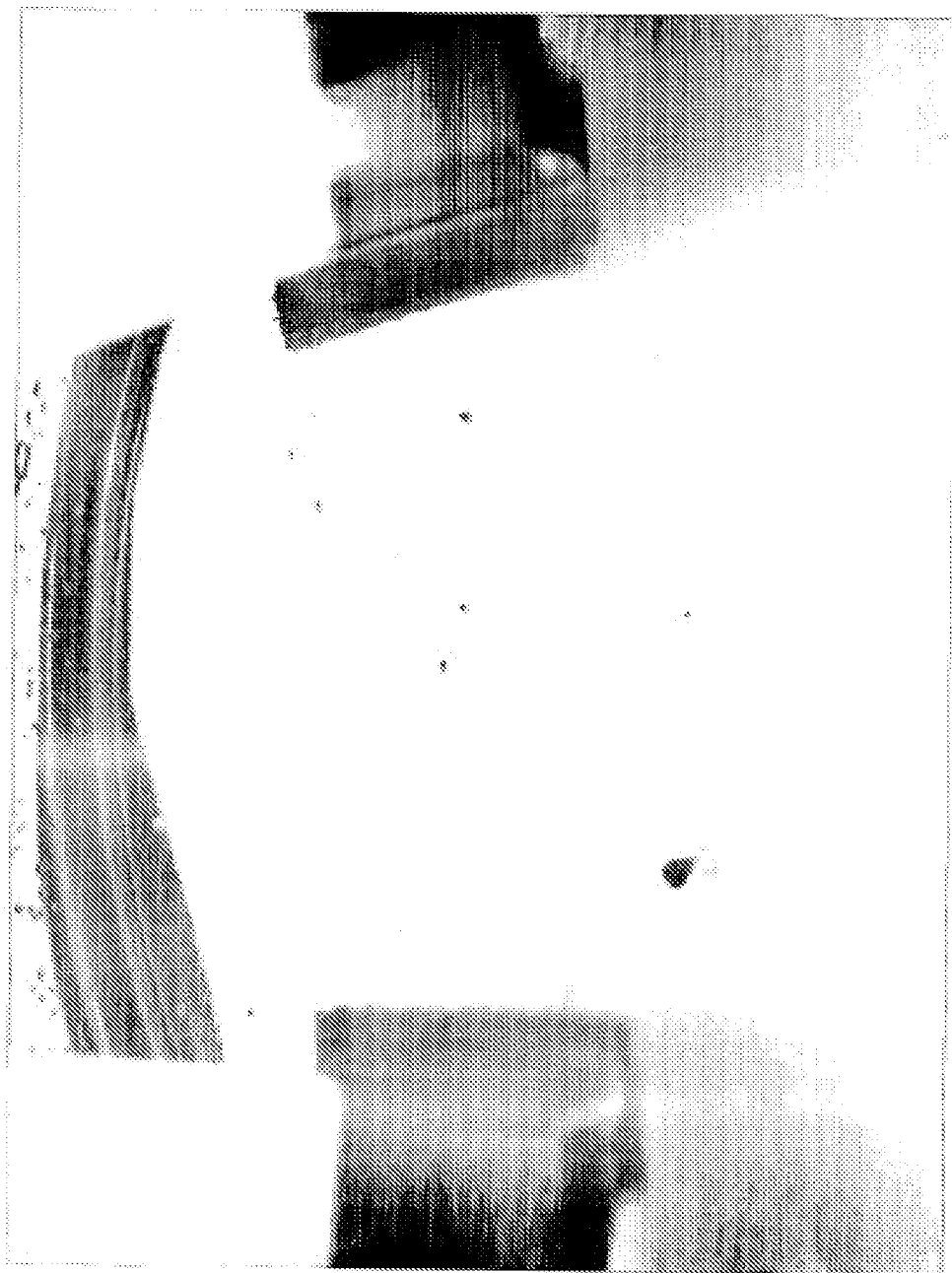
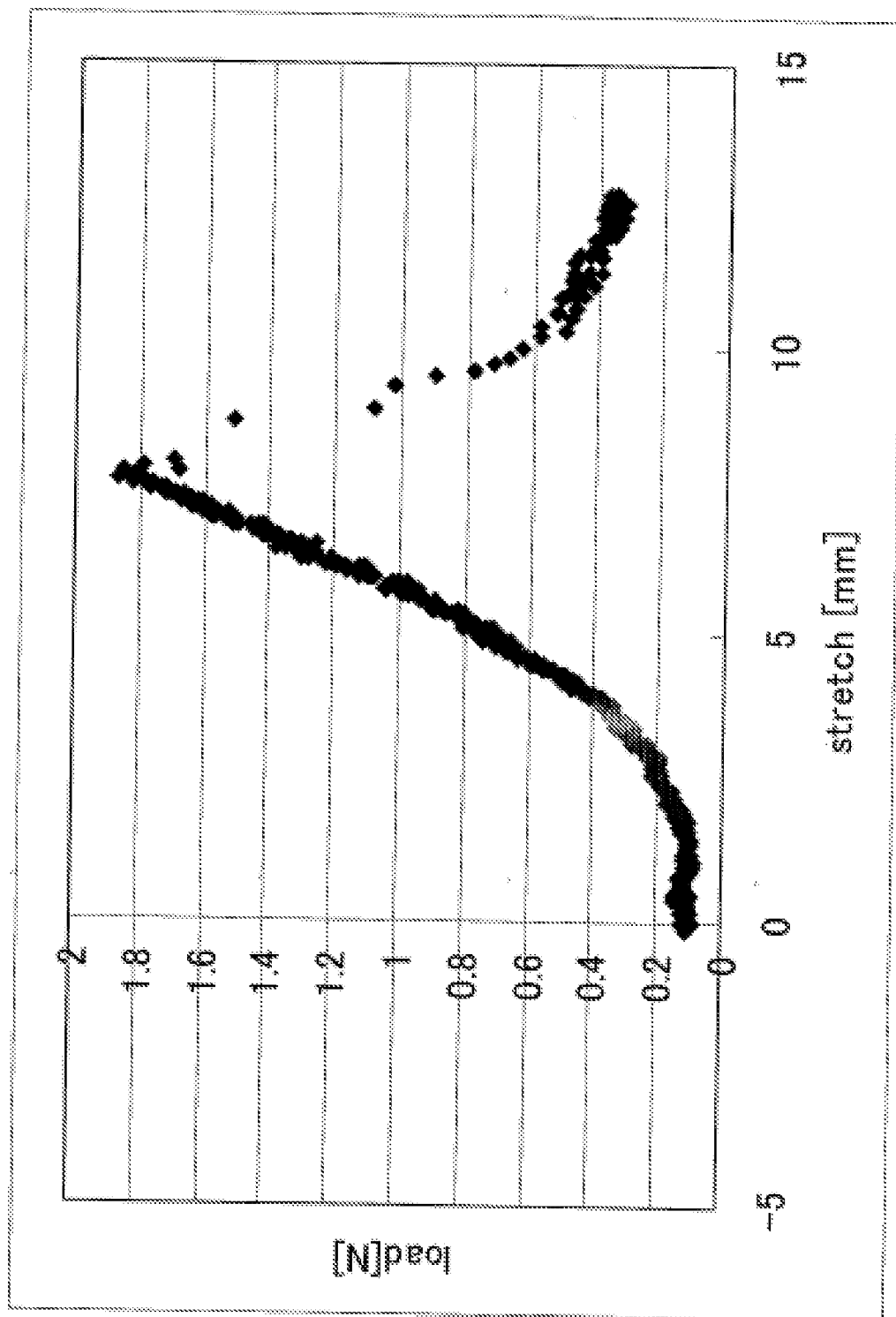


FIG.15

FIG.16



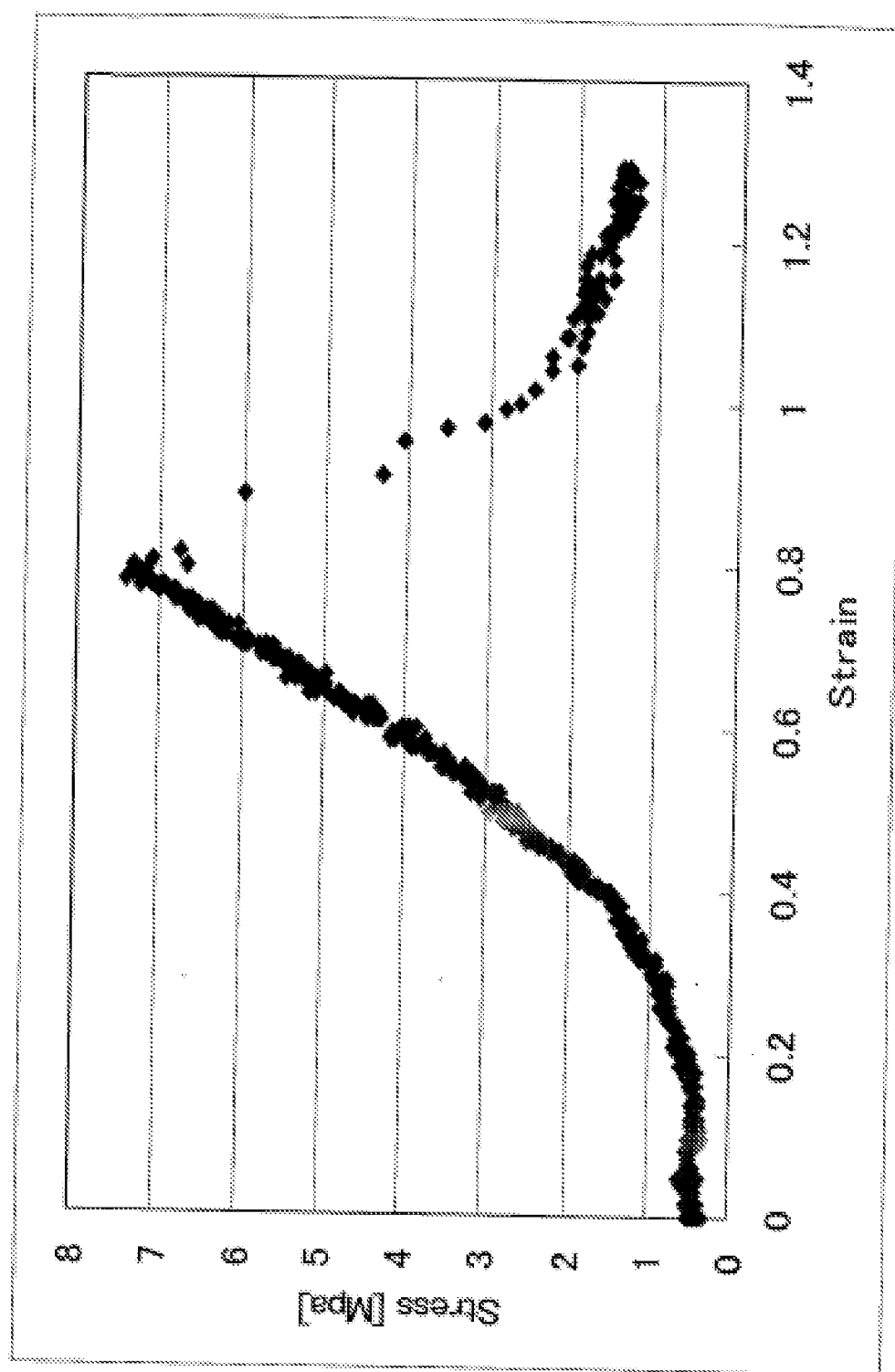


FIG.17

FIG.18

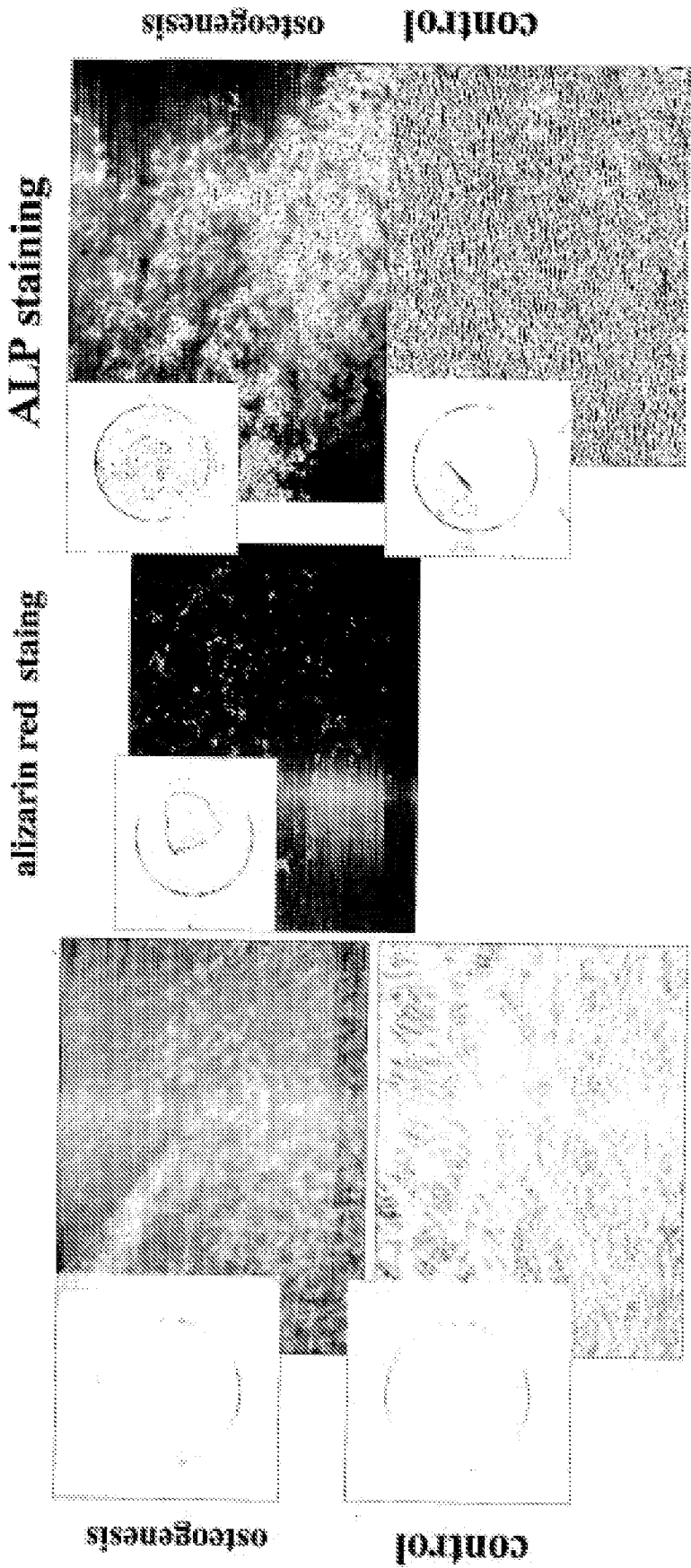
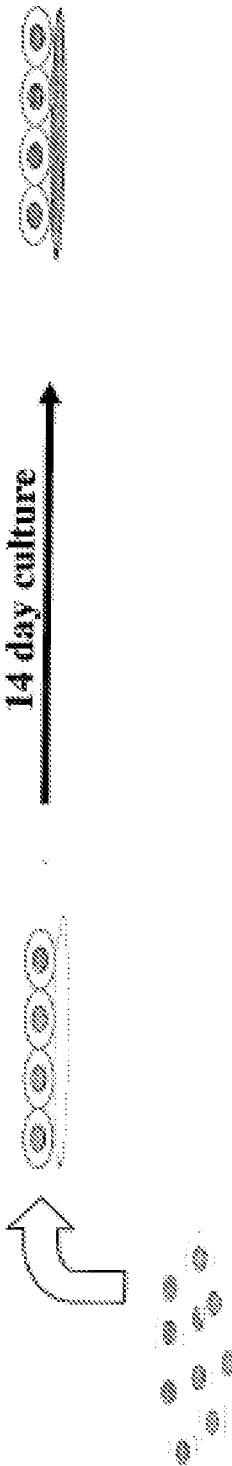
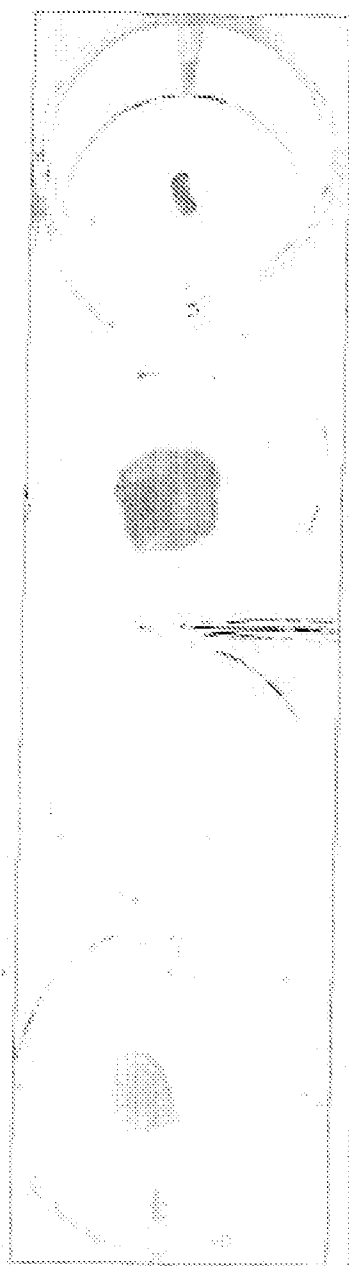


FIG.19

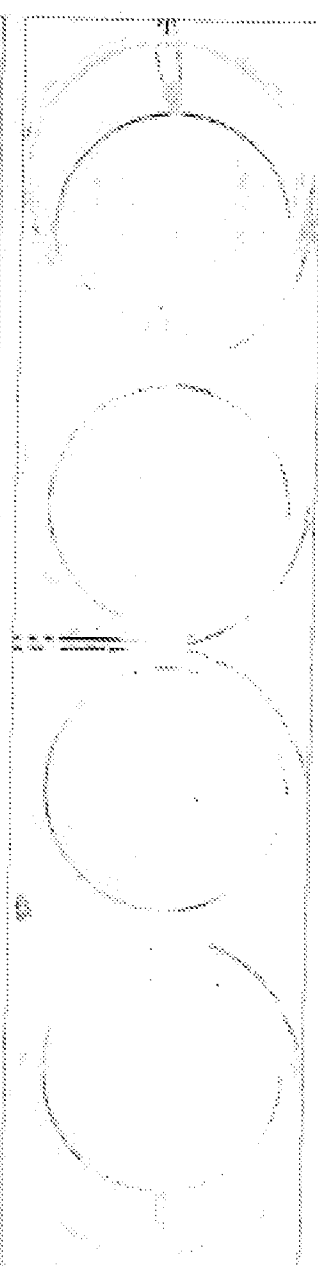
A)

Synthetic
tissue



B)

monolayer



Culture medium

Chondrogenic

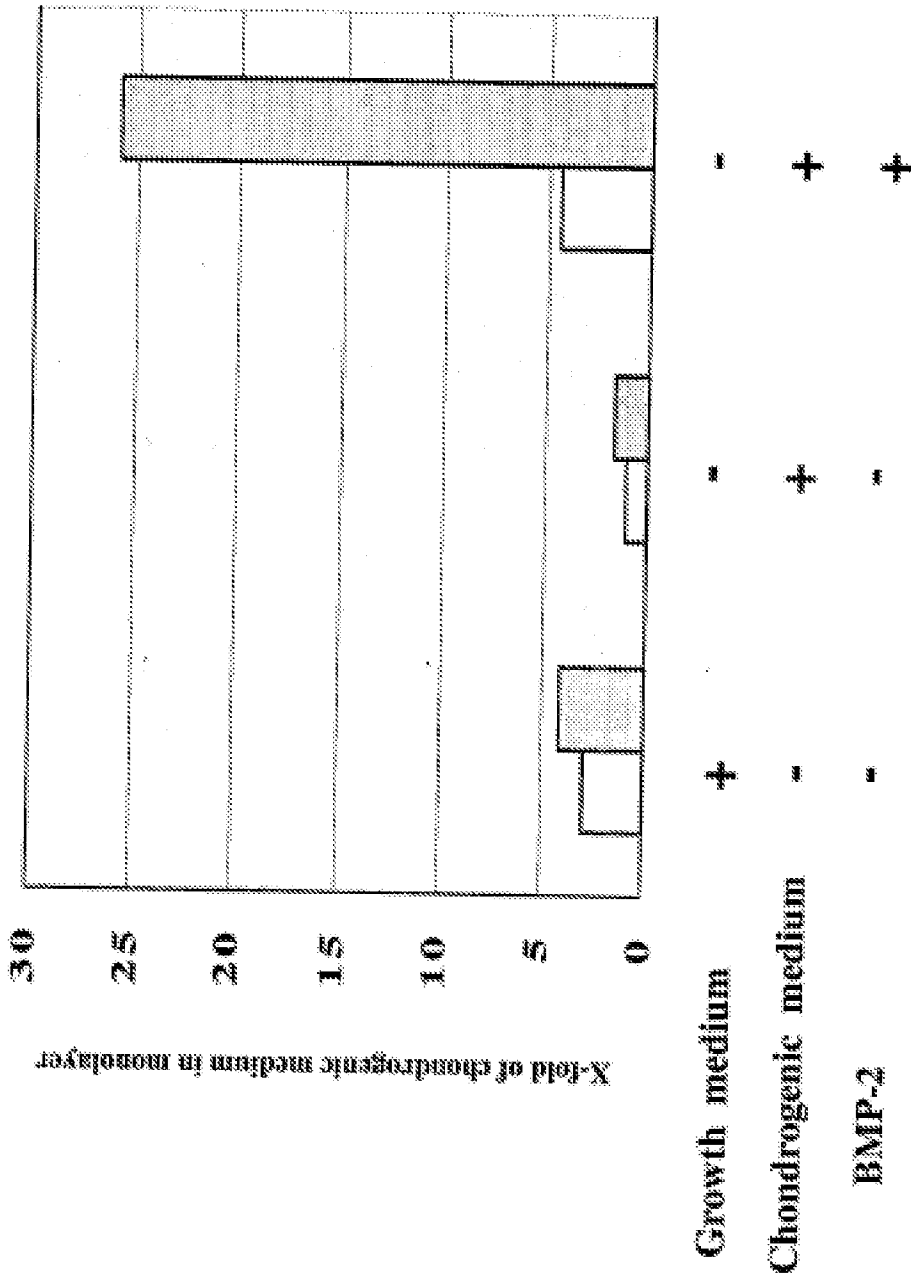
medium

BMP-2

TGF- β

+	-	-	-	-
-	+	+	+	+
-	-	-	-	-
-	-	-	-	-

FIG.20



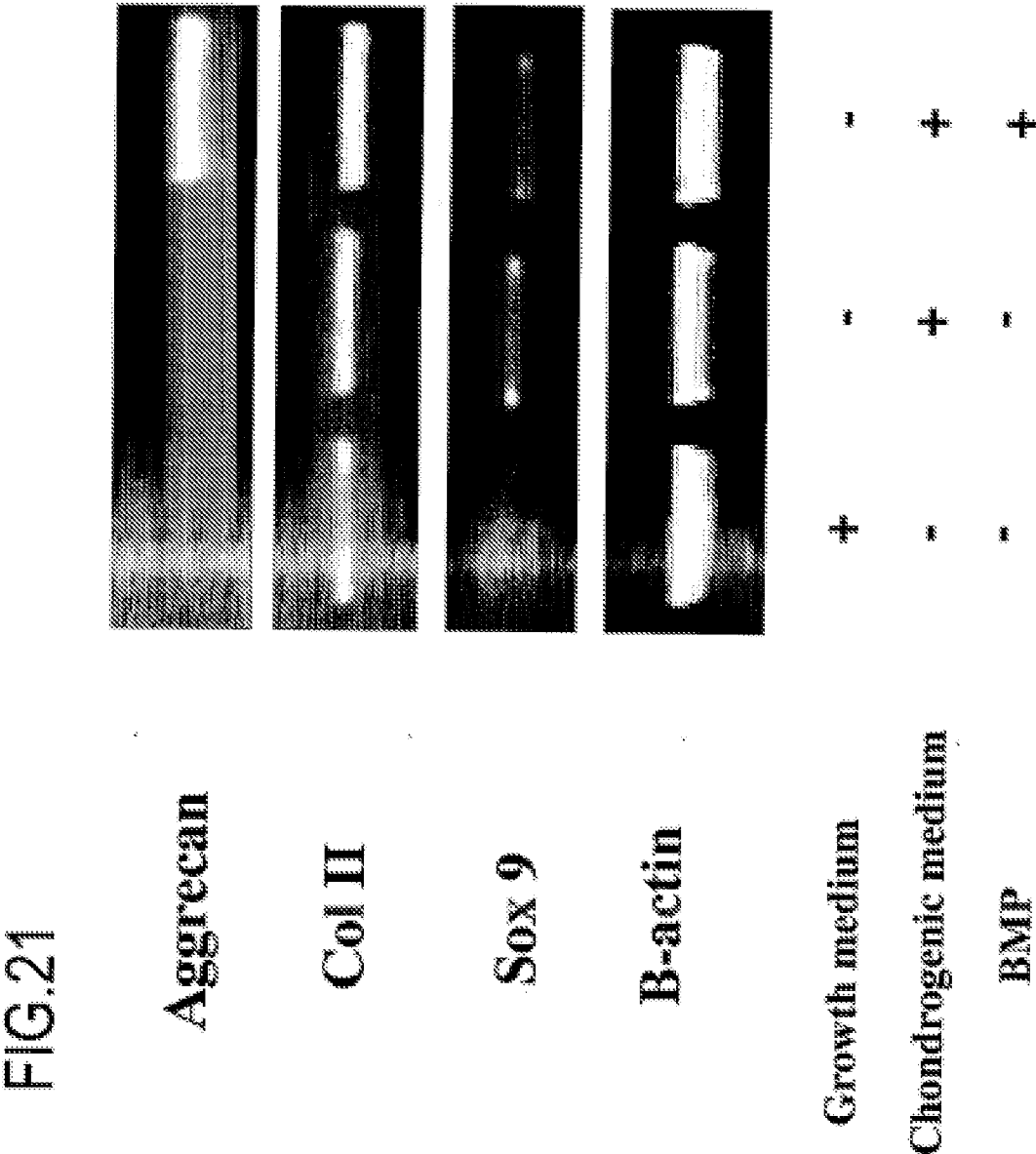


FIG.22

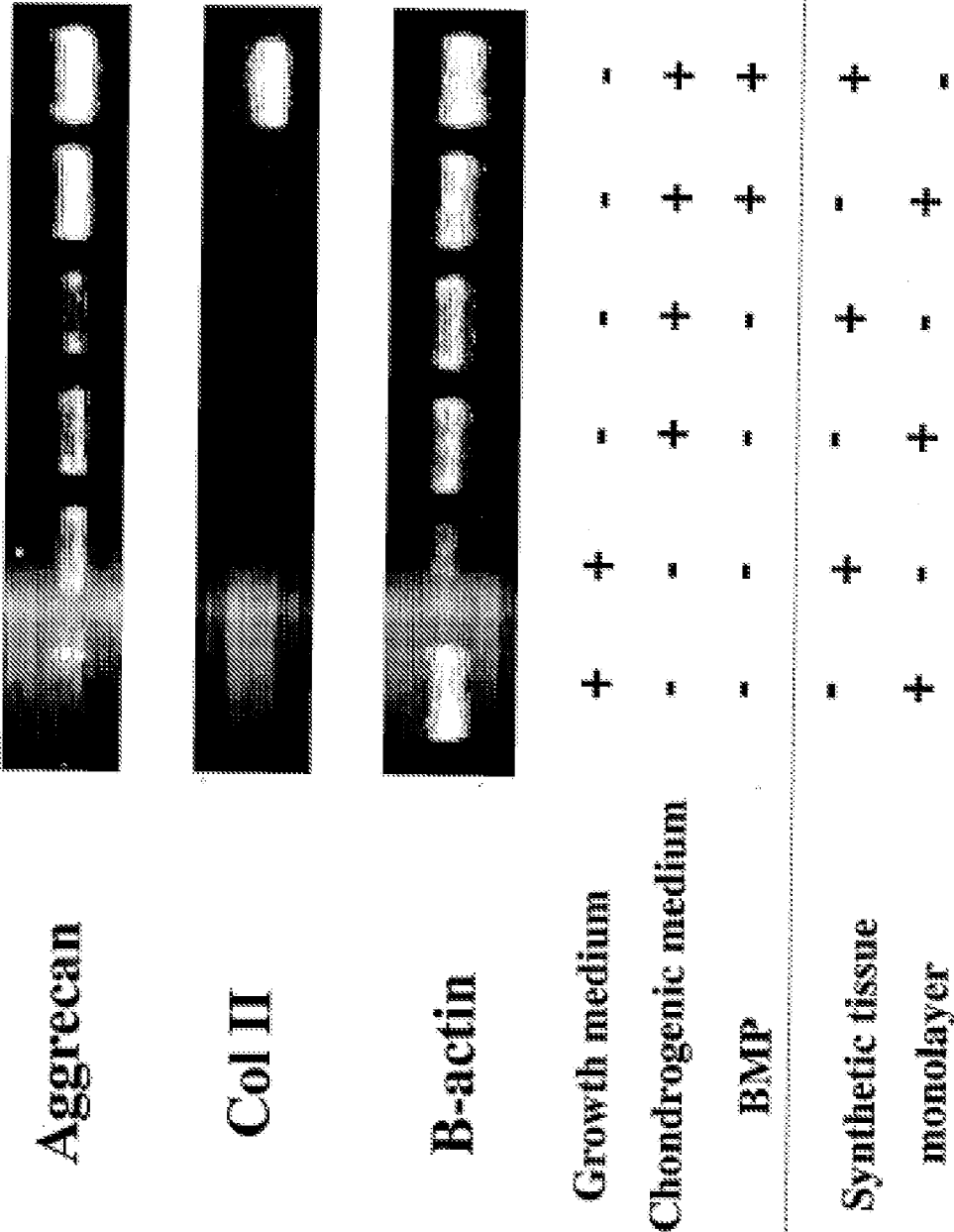
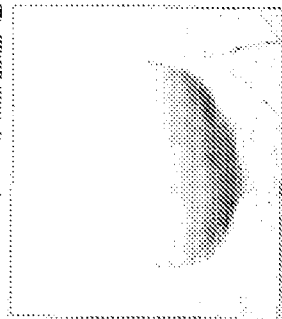


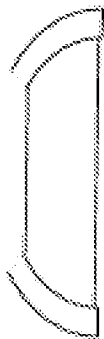
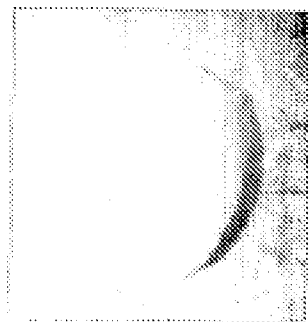
FIG.23

**remove superficial zone
digested with
chondroitinase ABC**



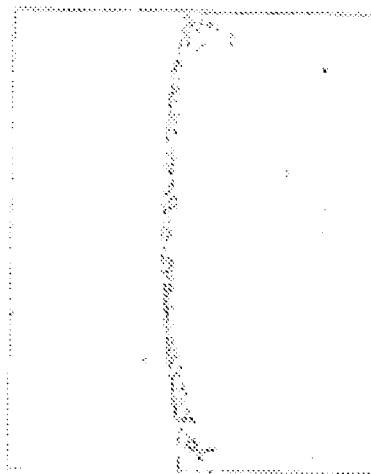
(Hunziker EB. JBJS 1996)

**Cultured for
7 days**

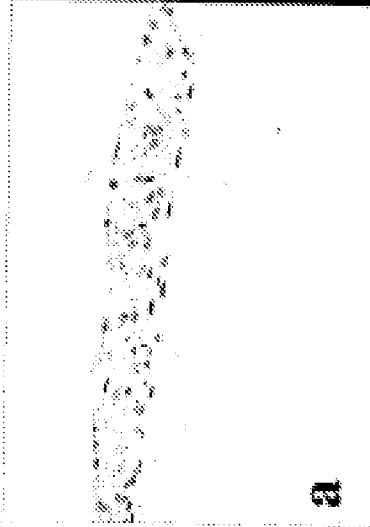


HE staining

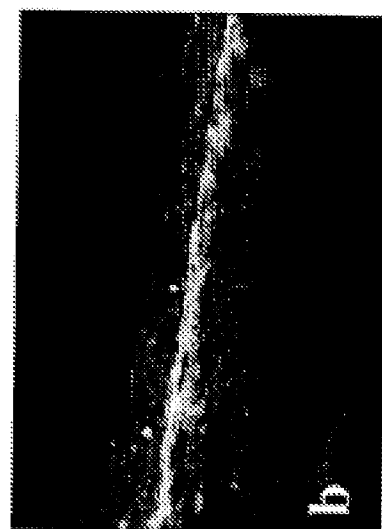
Fibronectin



x40



a



b

x200

FIG.24

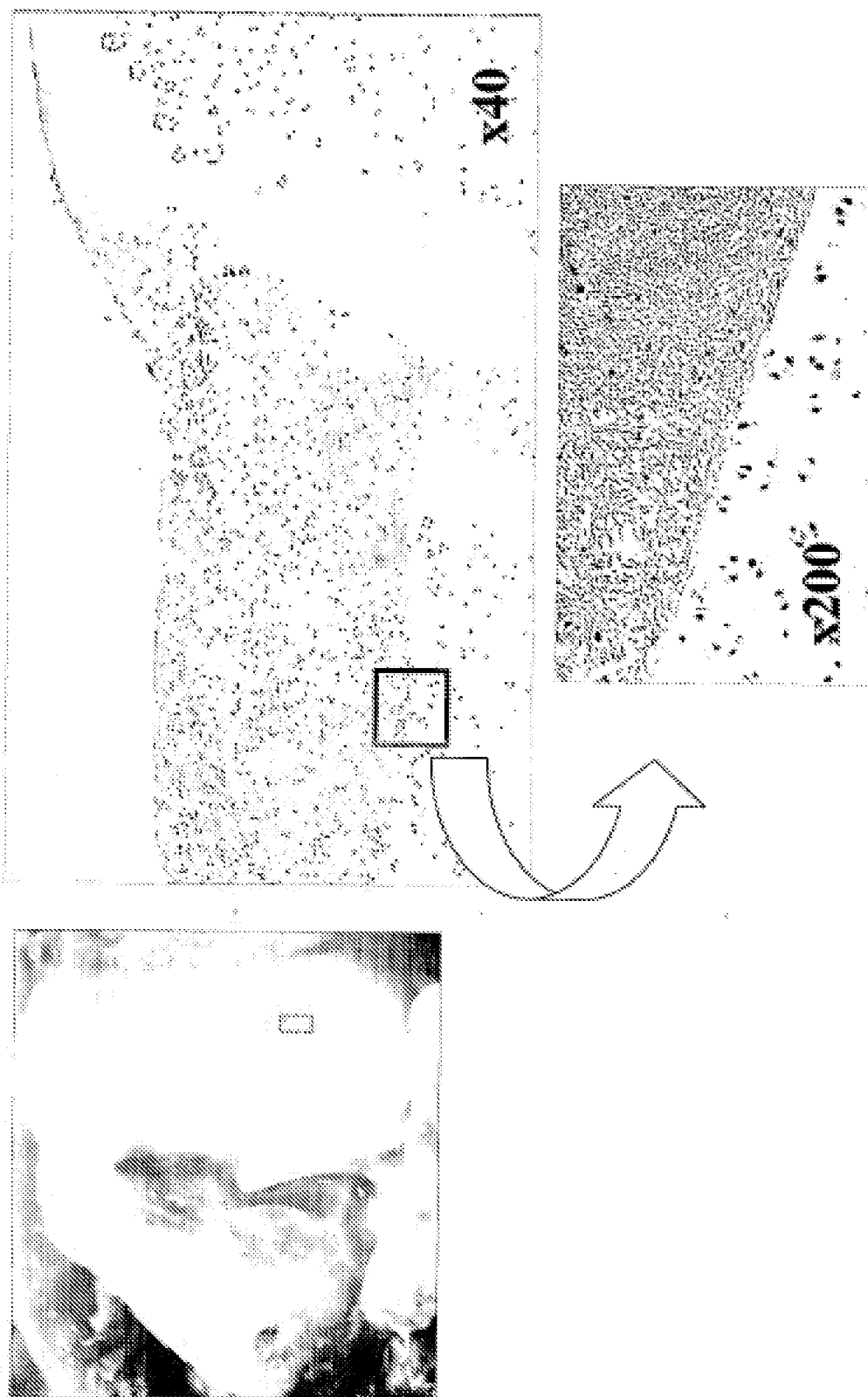


FIG.25

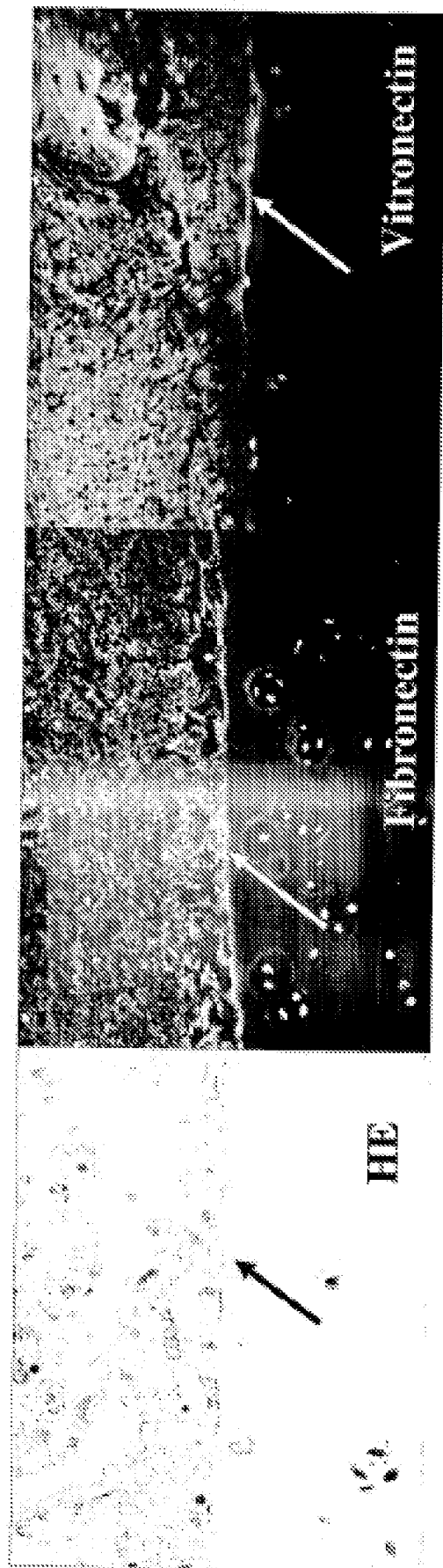


FIG.26

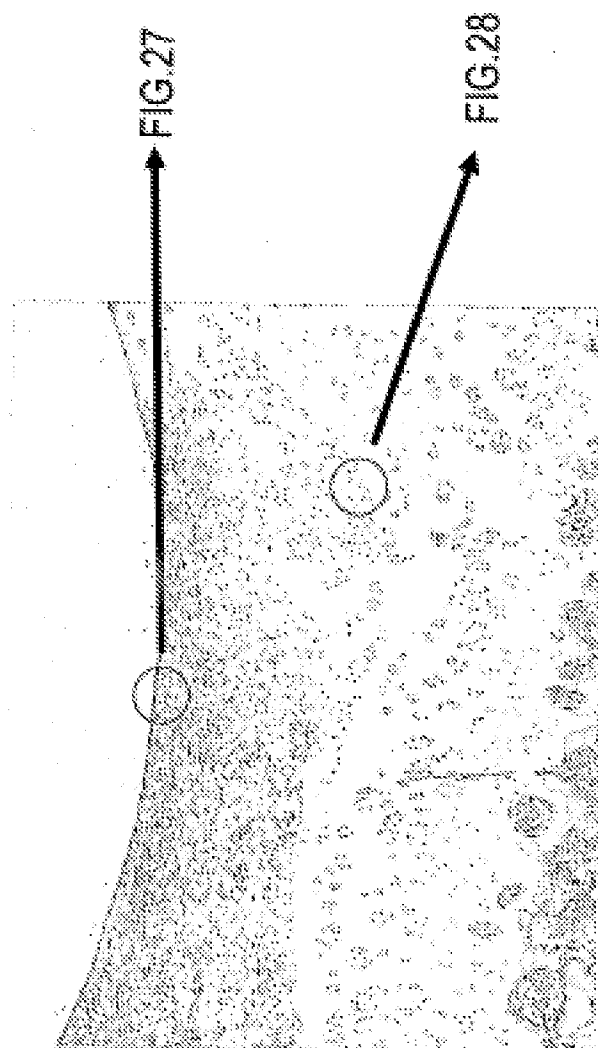


FIG.27

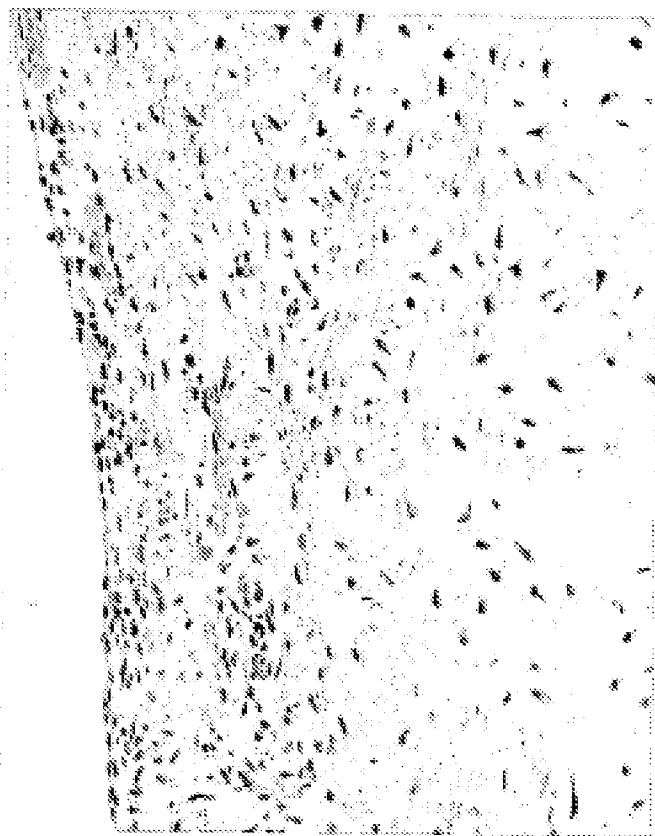


FIG.28



FIG.29

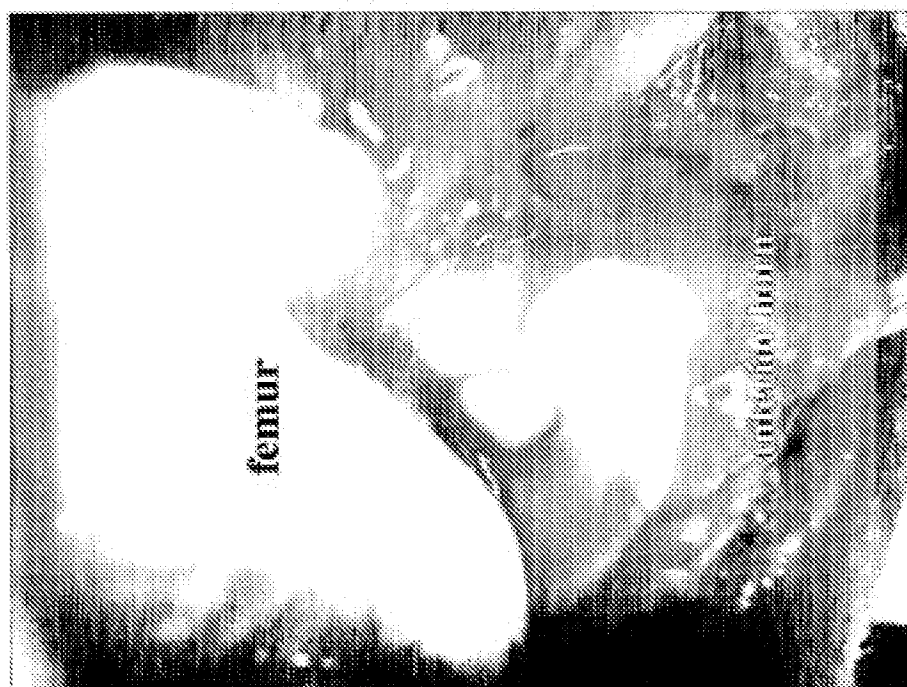
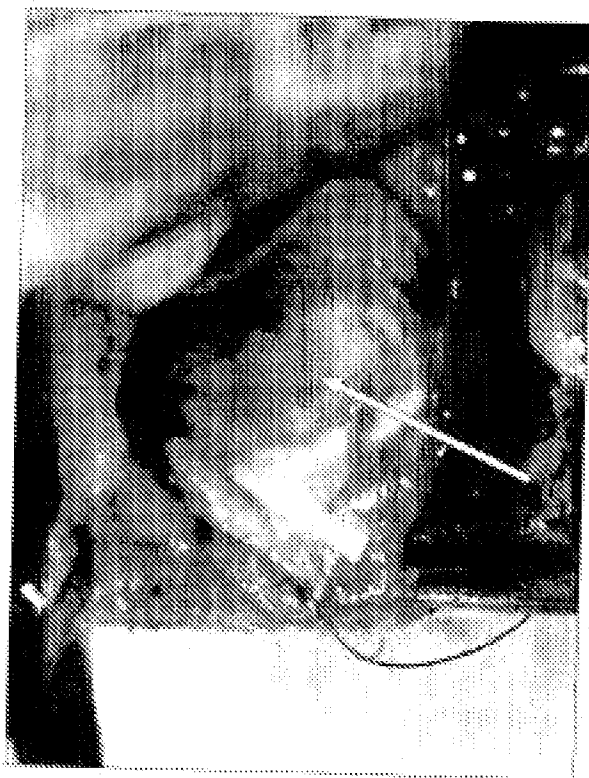
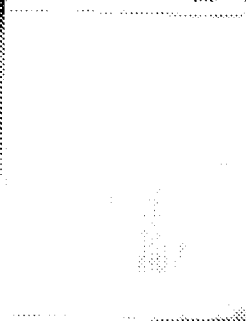


FIG.30



membrana synovialis
derived artificial tissue



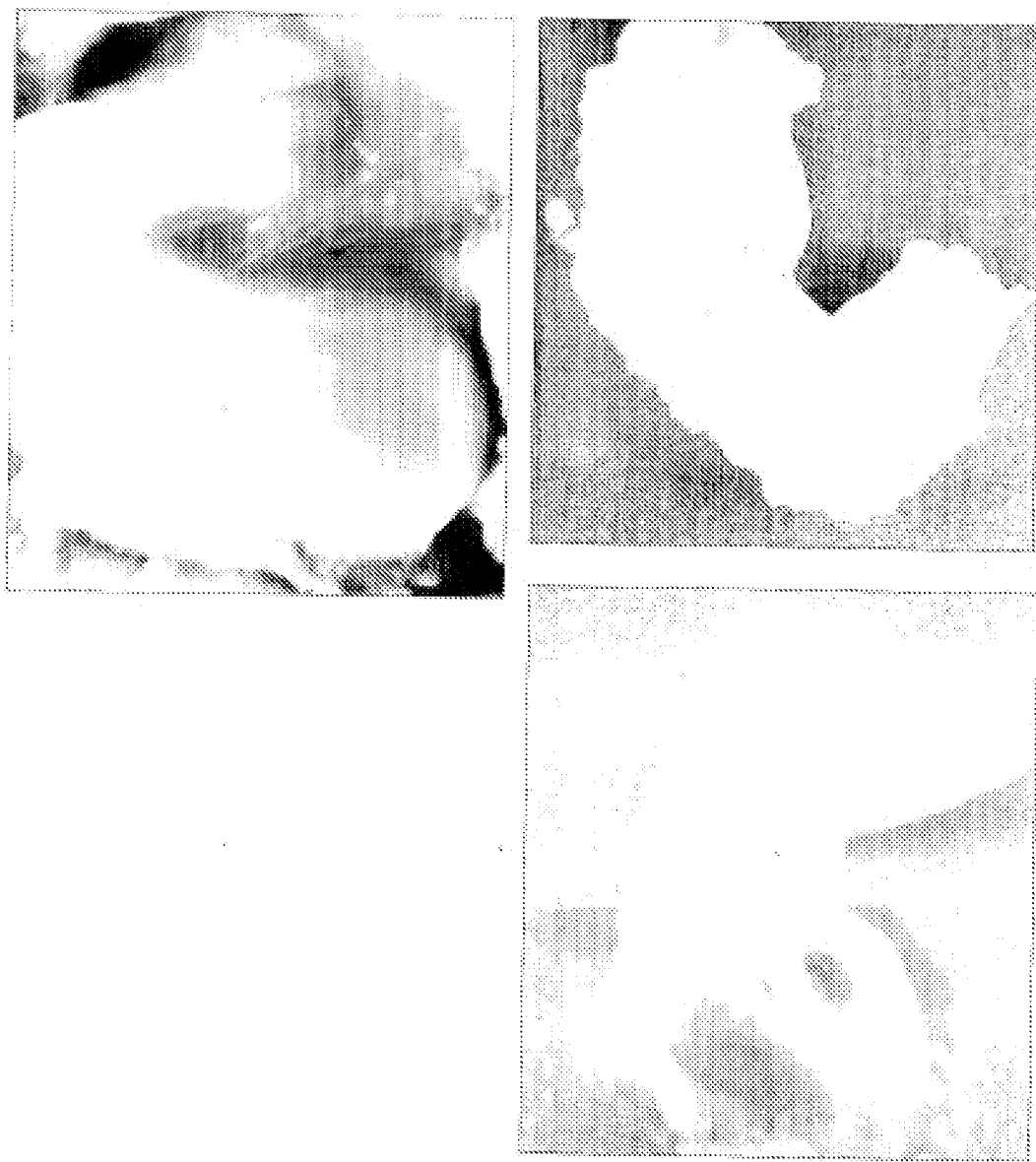


FIG.31

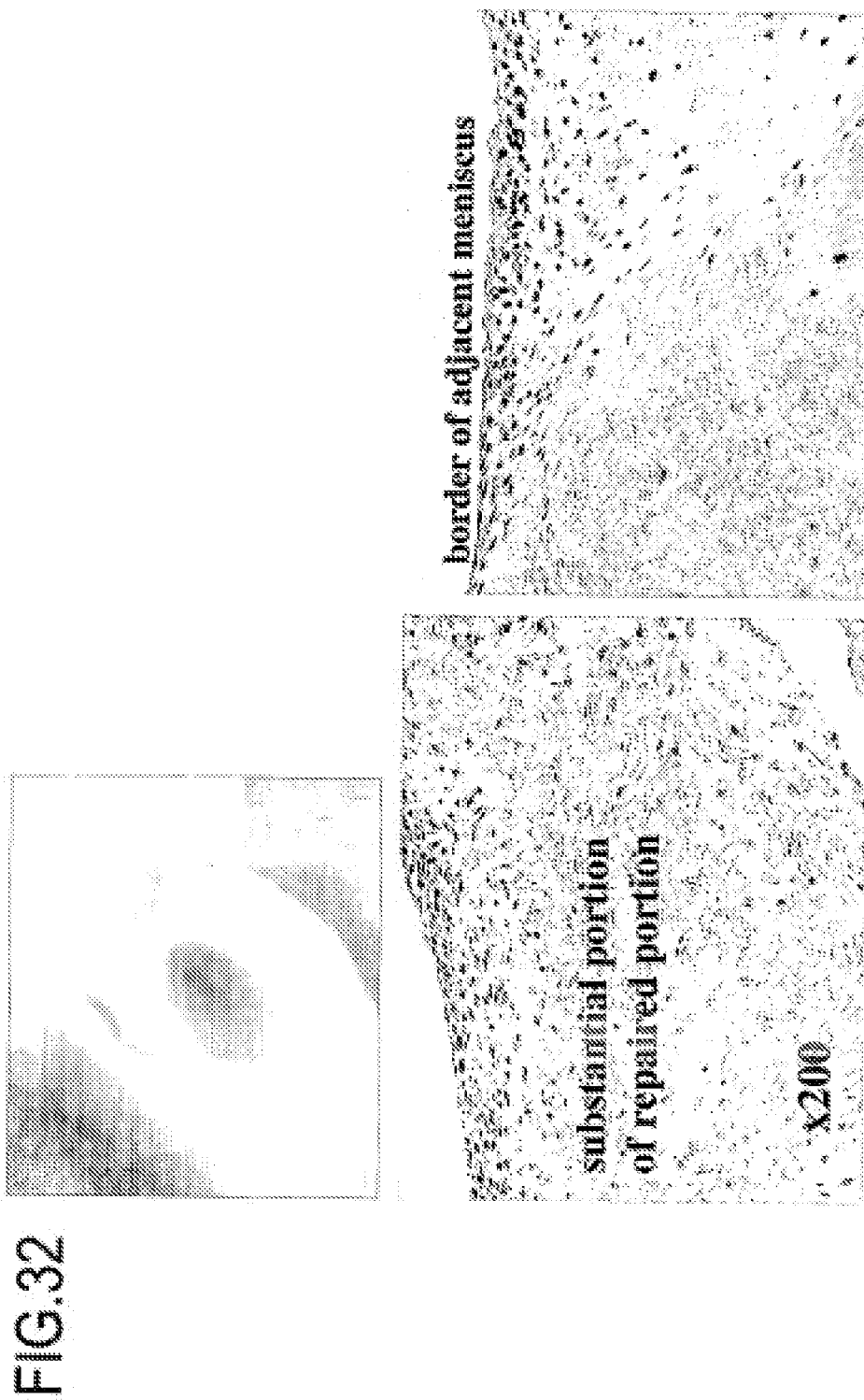
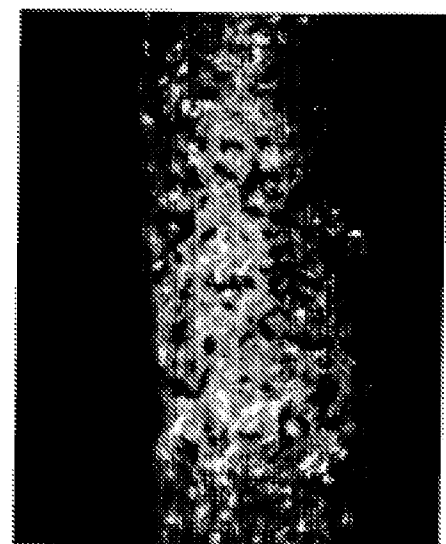
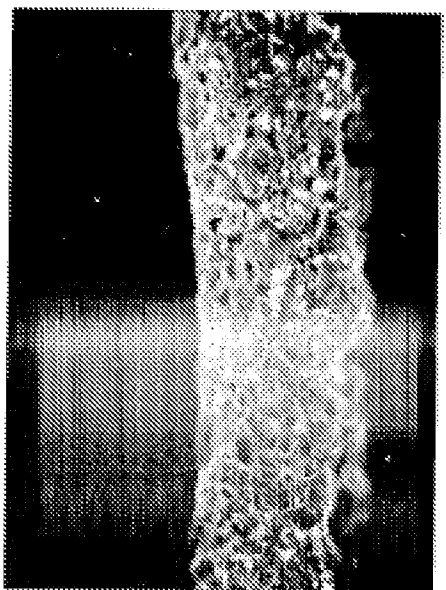


FIG.33



Vitronectin



Fibronectin

HE staining

FIG.34

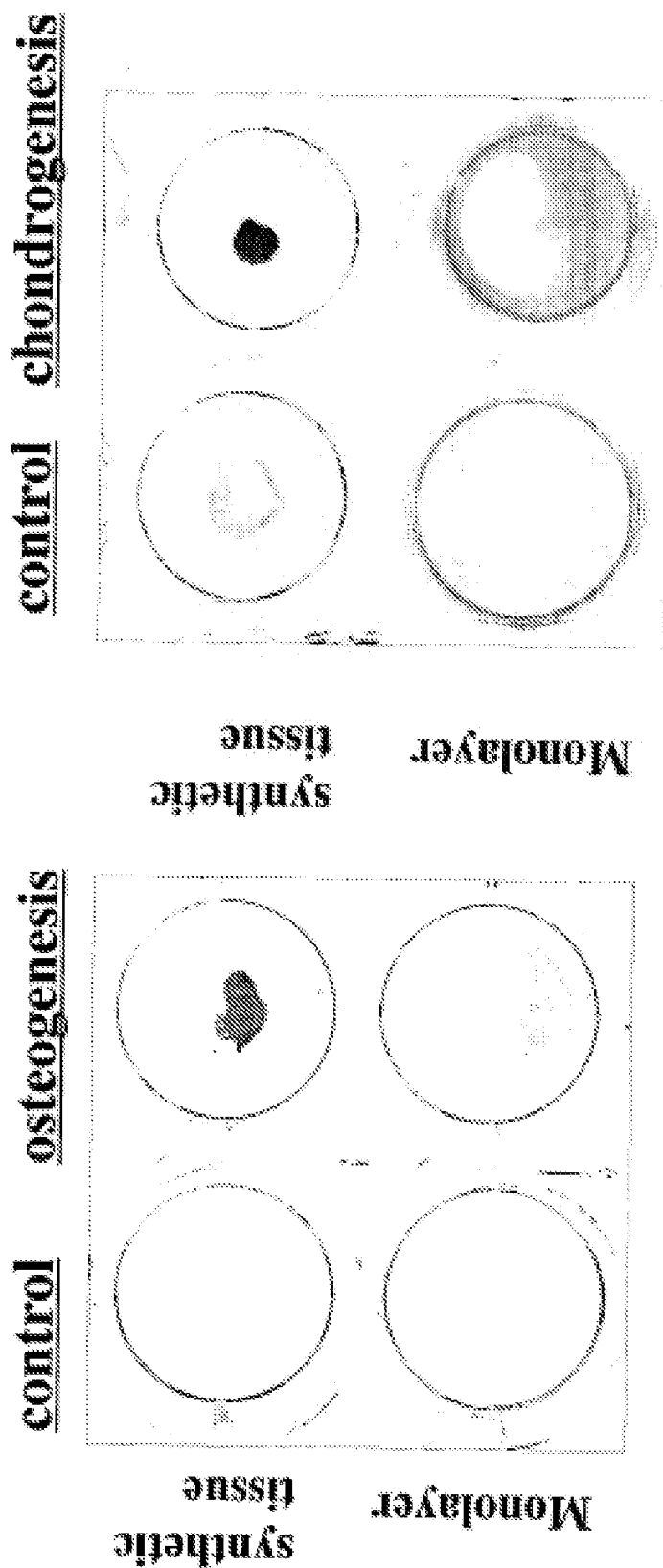


FIG.35

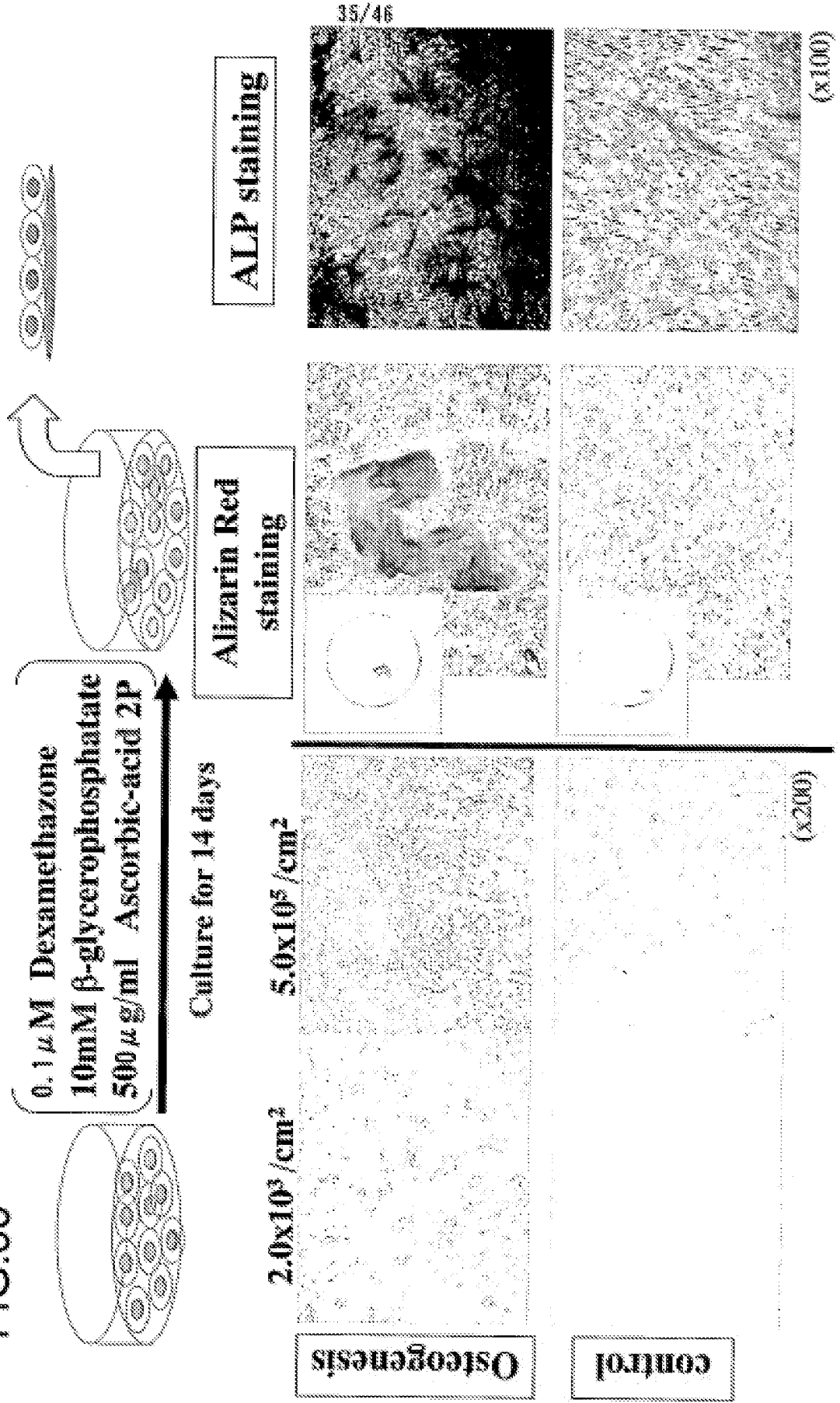


FIG.36

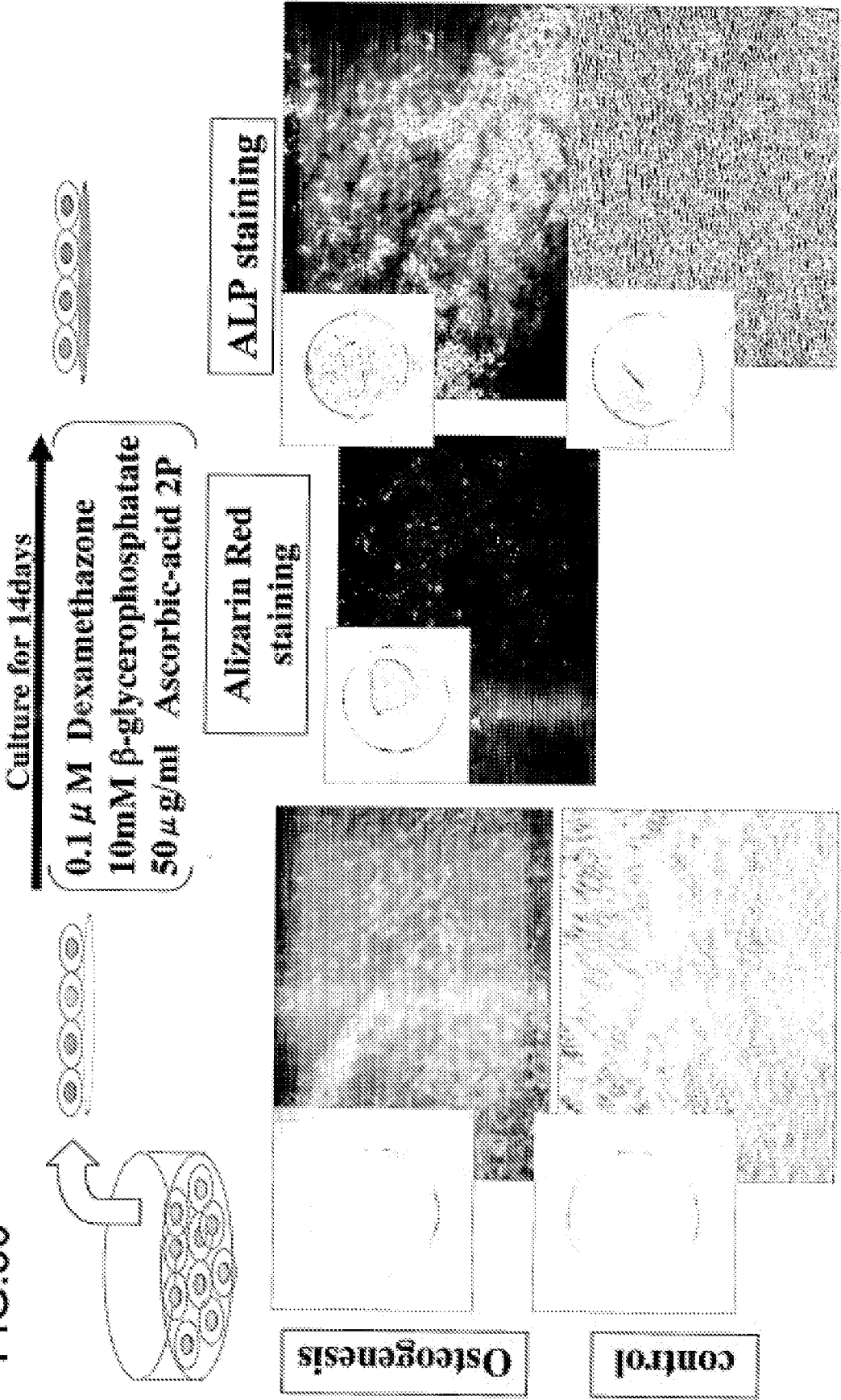
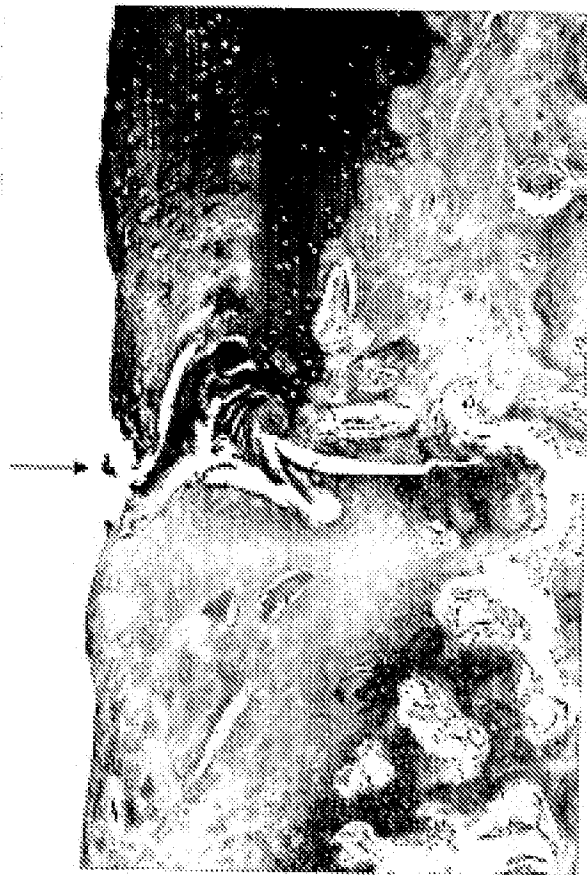
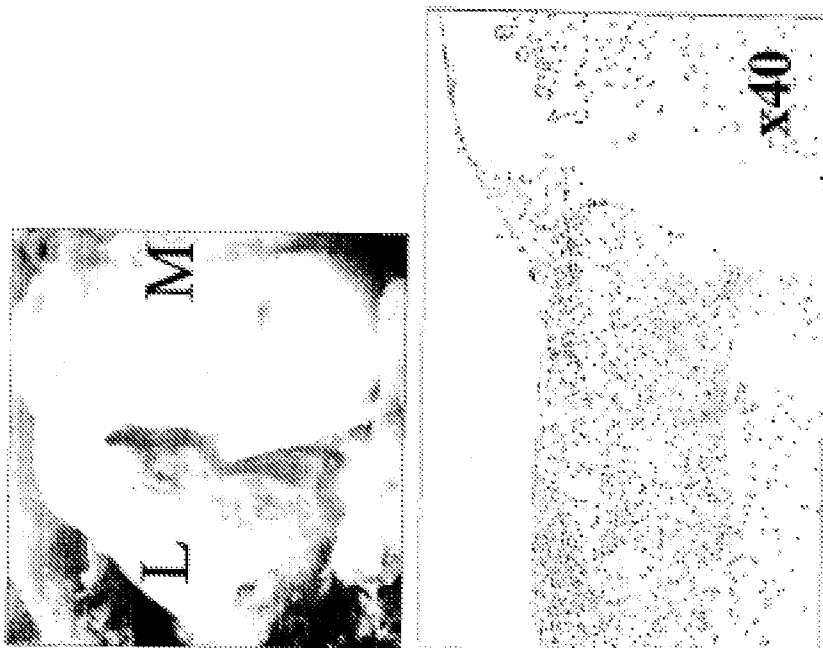


FIG.37



38/46



10days ↑



Post-o-pe



Pre-o-pe

FIG.38

FIG.39



FIG.40

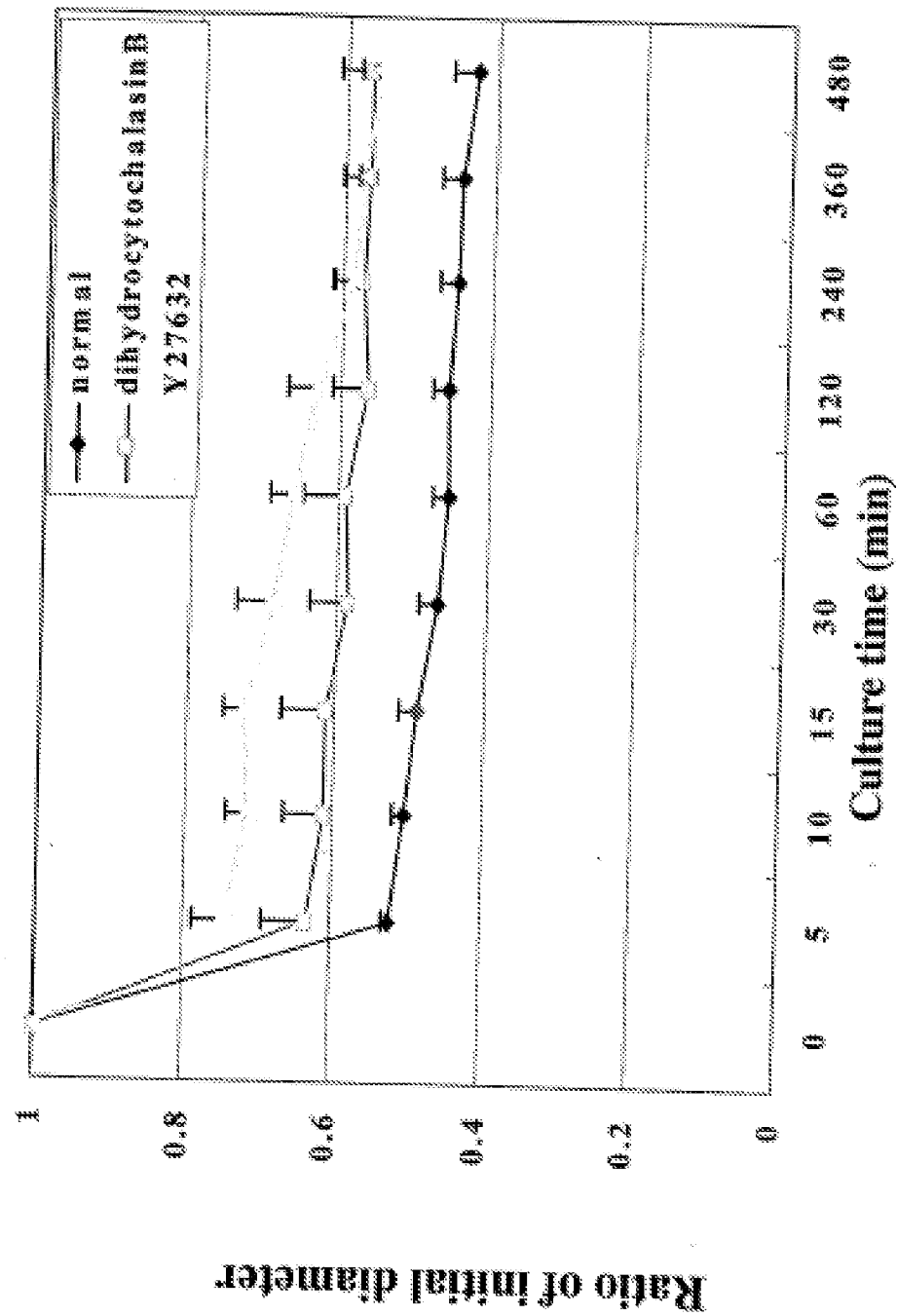


FIG.41

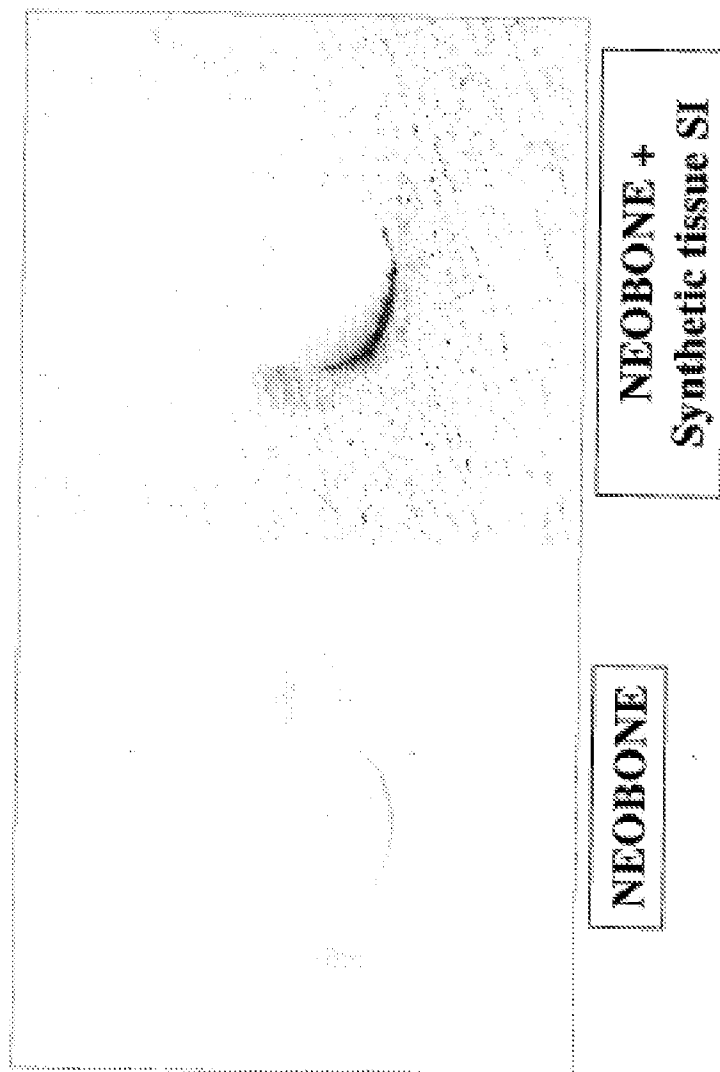


FIG.42

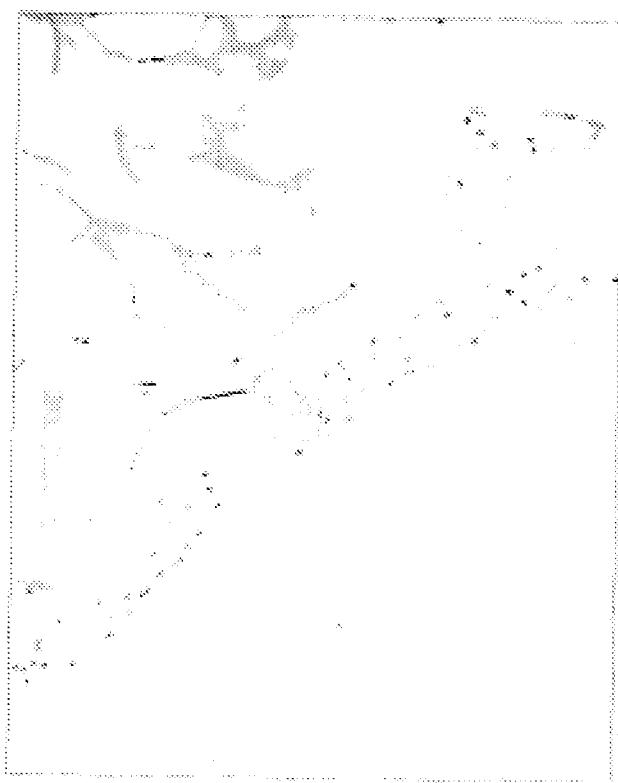


FIG.43

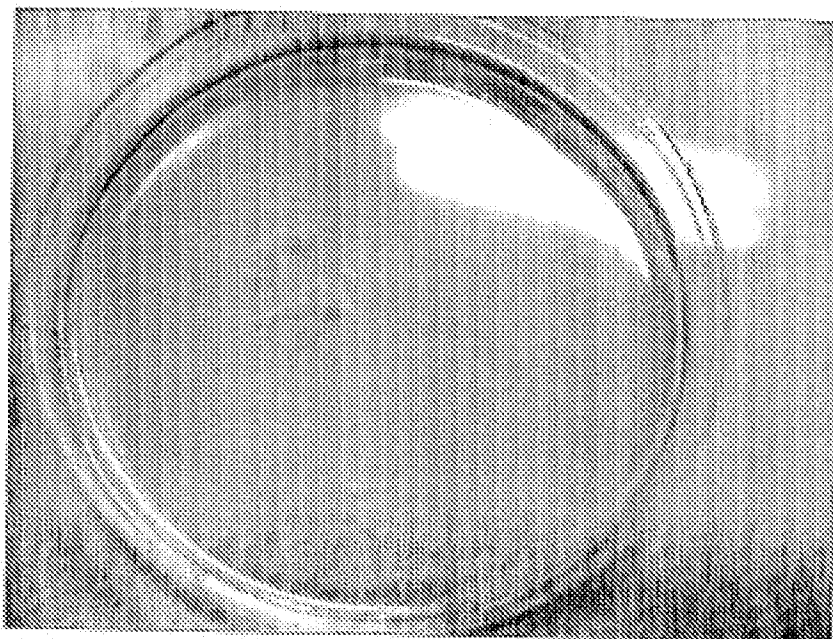


FIG.44

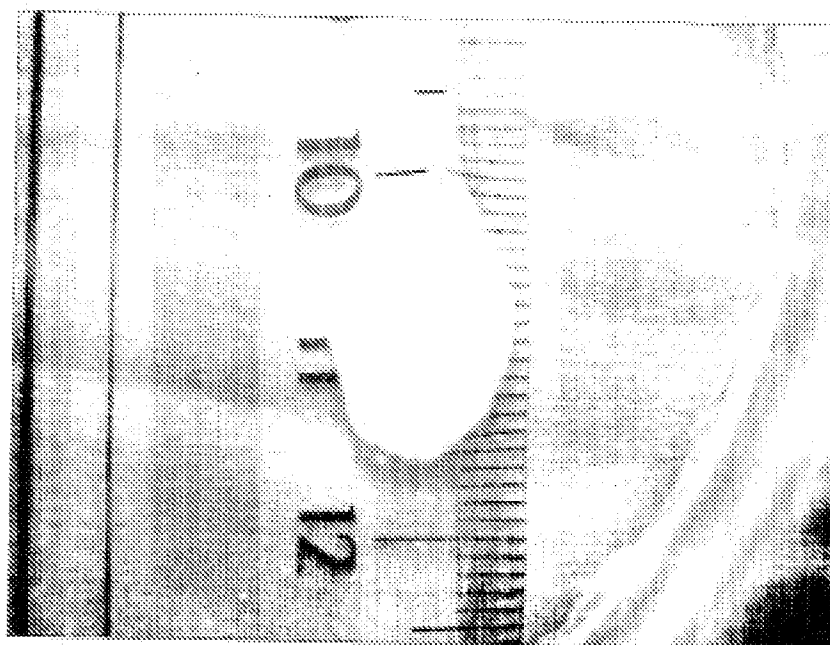
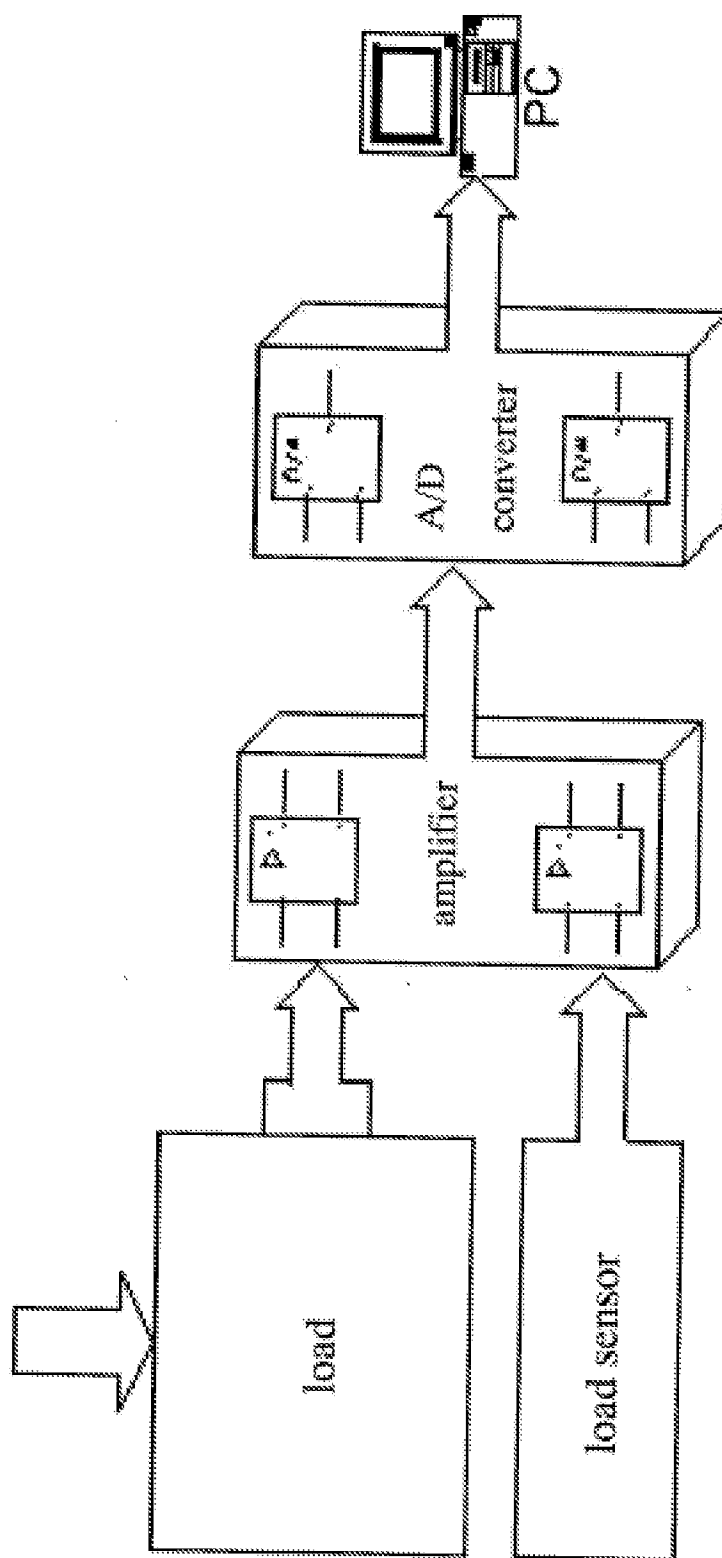
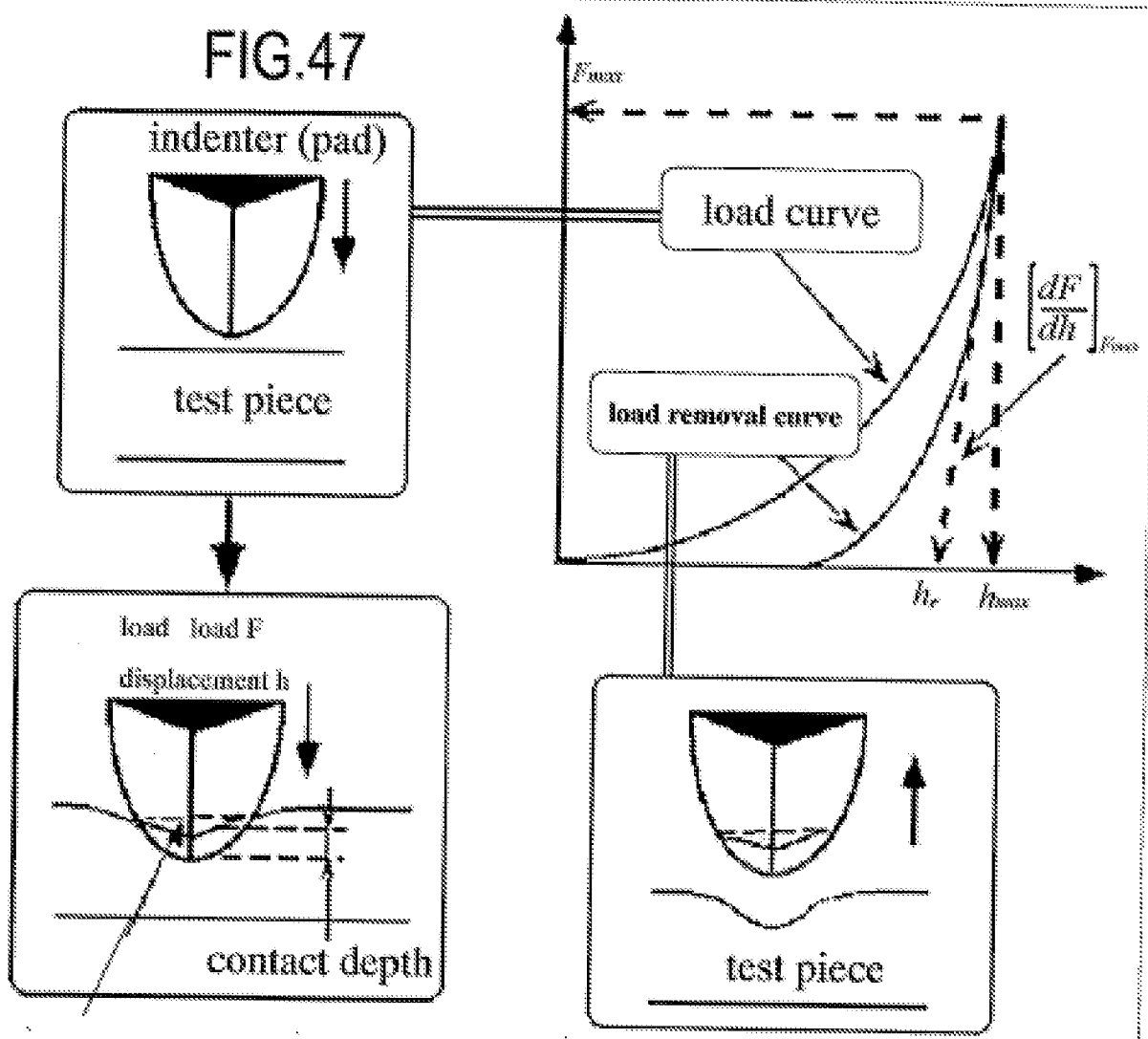


FIG.45



FIG.46





contact
projection A

$$H = \frac{F}{A} = \frac{F}{k_1 h_p^2}$$

$$E = \left[\frac{dF}{dh} \right]_{F_{max}} \frac{1 - \nu^2}{2 \cdot k_2 \cdot h_{pmax}}$$

$$h_p = h_r + 0.25(h_{max} - h_r)$$

F : load

A : contact projection area

h_p : contact depth

$k_1 k_2$: shape conflict

F_{max} : Maximum load

h_{max} : Maximum displacement

h_r : point at which tangential
line intersects

dF/dh : Gradient of tangential
line of load removal curve

ν : Poisson's ratio

SEQUENCE LISTING

<110> NAKANURA, Norimasa; MATSUDA, Hikaru; SAWA, Yoshiaki; TAKETANI, Satoshi; MIYAGAWA, Shigeru; YOSHIKAWA, Hideki; ANDO, Wataru

<120> SCAFFOLD-FREE SELF-ORGANIZED 3D SYNTHETIC TISSUE

<130> NKND01PCT

<160> 30

<170> PatentIn version 3.2

<210> 1

<211> 6085

<212> DNA

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<220>

<221> CDS

<222> (115)..(5940)

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actccacgga tacatcttct cacttgctaa caagggccto tgagtcagc agcc atg 117

Met

1

agt tca gac tca gaa ttg gct att ttt ggg gag gct gct cct ttc ctc 165

Ser Ser Asp Ser Glu Leu Ala Val Phe Gly Glu Ala Ala Pro Phe Leu

5

10

15

cga aag tct gaa aag gag cgc att gag gcc gag aat agc ccc ttt gat 213

Arg Lys Ser Glu Arg Glu Arg Ile Glu Ala Gln Asn Arg Pro Phe Asp

20	25	30	
gac aac aca tct gtc ttt gta gag gcc aac gaa tcc ttt gtc aac			261
Ala Lys Thr Ser Val Phe Val Ala Glu Pro Lys Glu Ser Phe Val Lys			
35	40	45	
ggg acc atc cag agc aga gaa gaa gaa aac gta acc gta aag act gag			309
Gly Thr Ile Gln Ser Arg Glu Gly Gly Lys Val Thr Val Lys Thr Glu			
50	55	60	65
gaa gaa gag act ctg aca gta aag gat gat cag gtc ttc ccc atg aac			357
Gly Gly Ala Thr Leu Thr Val Lys Asp Asp Gln Val Phe Pro Met Asn			
70	75	80	
cct ccc aac tat gac aag atc gag gat atg gcc atg atg act cat ctg			405
Pro Pro Lys Tyr Asp Lys Ile Glu Asp Met Ala Met Met Thr His Leu			
85	90	95	
cat gag cct gat gta ctg tac aac atc aac gaa cgt tat gca gcc tgg			453
His Glu Pro Ala Val Leu Tyr Asn Leu Lys Glu Arg Tyr Ala Ala Trp			
100	105	110	
atg atc tac acc tat tca ggt ctg ttc tgt gtc act gtc aac ccc tac			501
Met Ile Tyr Thr Tyr Ser Gly Leu Phe Cys Val Thr Val Asn Pro Tyr			
115	120	125	
aag tgg ctg cct gta tat aag ccc gag gta gta aca gcc tac cga ggc			549
Lys Trp Leu Pro Val Tyr Lys Pro Glu Val Val Thr Ala Tyr Arg Gly			
130	135	140	145
aac aag agc cag ggg gcc ccg ccc ccc atc ttc tcc atc tat gac aac			597
Lys Lys Arg Gln Gly Ala Pro Pro His Ile Phe Ser Ile Ser Asp Asn			
150	155	160	
gcc tat cag ttc atg ctg act gac cga gag aat cag tca atc ctg atc			645
Ala Tyr Gln Phe Met Leu Thr Asp Arg Glu Asn Gln Ser Ile Leu Ile			

165	170	175	
act gaa gaa tct ggt gca ggg aag act gtg aac acc aag cgt gtc atc			693
Thr Gly Glu Ser Gly Ala Gly Lys Thr Val Asn Thr Lys Arg Val Ile			
180	185	190	
cag tac ttt gca aca att gca gtt act ggt gag aag aag aag gaa gaa			741
Gln Tyr Phe Ala Thr Ile Ala Val Thr Gly Glu Lys Lys Lys Glu Glu			
195	200	205	
att act tct ggc aaa ala cag gag act ctg gaa gat caa atc atc agt			789
Ile Thr Ser Gly Lys Ile Gln Gly Thr Leu Glu Asp Gln Ile Ile Ser			
210	215	220	225
gca aac acc cta ctg gag gcc ttt ggc aac gcc aag acc gtg agg aat			837
Ala Asn Pro Leu Leu Glu Ala Phe Gly Asn Ala Lys Thr Val Arg Asn			
230	235	240	
gac aac tcc tct cgc ttt ggt aaa ttc atc aga atc cac ttt ggc act			885
Asp Asn Ser Ser Arg Phe Gly Lys Phe Ile Arg Ile His Phe Gly Thr			
245	250	255	
act gga aaa ctg gca tct gct gat att gaa aca tat ctg cta gag aag			933
Thr Gly Lys Leu Ala Ser Ala Asp Ile Glu Thr Tyr Leu Leu Glu Lys			
260	265	270	
tct aga gtt gtt ttc cag ctt aag gct gag aga agt tat cat att ttt			981
Ser Arg Val Val Phe Gln Leu Lys Ala Glu Arg Ser Tyr His Ile Phe			
275	280	285	
tac cag att aca tog aet aag aaa cca gaa ctt att gaa atg ctt ctg			1029
Tyr Gln Ile Thr Ser Asn Lys Lys Pro Glu Leu Ile Glu Met Leu Leu			
290	295	300	305
att acc aag aac cca tat gat tac cca ttt gtc agt caa gag gag atc			1077
Ile Thr Thr Asn Pro Tyr Asp Tyr Pro Phe Val Ser Gln Gly Glu Ile			

310	315	320	
agt gtg gcc agc atc gat gat cag gaa gaa ctg atg gcc aca gat agt Ser Val Ala Ser Ile Asp Asp Gln Glu Glu Leu Met Ala Thr Asp Ser			1125
325	330	335	
gct att gat att ttg ggc ttt act aat gaa gaa aag gtc tcc att tac Ala Ile Asp Ile Leu Gly Phe Thr Asn Glu Glu Lys Val Ser Ile Tyr			1173
340	345	350	
aag ctc aag gag gct gtg atg cat tat gag aac cta aaa ttt aag caa Lys Leu Thr Gly Ala Val Met His Tyr Gly Asn Leu Lys Phe Lys Gln			1221
355	360	365	
aag cag cgt gag gag caa gca gag cca gat ggc aca gaa gtt gct gac Lys Gln Arg Glu Glu Gln Ala Glu Pro Asp Gly Thr Glu Val Ala Asp			1269
370	375	380	385
aag gag gcc tac ctc cag agt ctg aac tct gca gat ctg ctc aca gct Lys Ala Ala Tyr Leu Gln Ser Leu Asn Ser Ala Asp Leu Leu Lys Ala			1317
390	395	400	
ctc tgc tac ccc agg gtc aag gtc ggc aat gag tat gtc aca aaa ggc Leu Cys Tyr Pro Arg Val Lys Val Gly Asn Glu Tyr Val Thr Lys Gly			1365
405	410	415	
cag act gta gaa cag gtg tcc aac gca gta ggt gct ctg gcc aaa gcc Gln Thr Val Glu Gln Val Ser Asn Ala Val Gly Ala Leu Ala Lys Ala			1413
420	425	430	
gtc tac gag aag atg ttc ctg tag atg gtt gcc cgc atc aac cag cag Val Tyr Glu Lys Met Phe Leu Trp Met Val Ala Arg Ile Asn Gln Gln			1461
435	440	445	
ctg gcc aac aag cag ccc agg cag tac ttc atc gag gtc ttg gac att Leu Asp Thr Lys Gln Pro Arg Gln Tyr Phe Ile Gly Val Leu Asp Ile			1509

450	455	460	465	
gct gat ttt gag att ttt gat ttc aac agc ctg gag cag ctg tgc atc				1557
Ala Gly Phe Glu Ile Phe Asp Phe Asn Ser Leu Glu Gln Leu Cys Ile				
470	475	480		
aac ttc acc aat gag aac ctg caa cag ttt ttc aac cac cac atg ttc				1605
Asn Phe Thr Asn Glu Lys Leu Gln Gln Phe Phe Asn His His Met Phe				
485	490	495		
atg ctg gag cag gag gag tac aag aag gaa agc atc gag tgg acg ttc				1653
Val Leu Glu Gln Glu Glu Tyr Lys Lys Glu Gly Ile Glu Trp Thr Phe				
500	505	510		
atc gac ttc ggg atg gac ctg gct gcc tgc atc gag ctc atc gag aag				1701
Ile Asp Phe Gly Met Asp Leu Ala Ala Cys Ile Glu Leu Ile Glu Lys				
515	520	525		
cct atg ggc atc ttc tcc atc ctg gaa gag gag tgc atg ttc cct aag				1749
Pro Met Gly Ile Phe Ser Ile Leu Glu Glu Glu Cys Met Phe Pro Lys				
530	535	540	545	
gca acc gac acc tcc ttc aag aac aag ctg tat gac cag cac ctg ggc				1797
Ala Thr Asp Thr Ser Phe Lys Asn Lys Leu Tyr Asp Gln His Leu Gly				
550	555	560		
aag tct gcc aac ttc cag aag ccc aag gtg gtc aac ggc aag gcc gag				1845
Lys Ser Ala Asn Phe Gln Lys Pro Lys Val Val Lys Gly Lys Ala Glu				
565	570	575		
gcc cac ttc gct ctg att cac tat gct ggt gtt gtg gac tac aac att				1893
Ala His Phe Ala Leu Ile His Tyr Ala Gly Val Val Asp Tyr Asn Ile				
580	585	590		
act gcc tgg ctg gag aag aac aag gac ccc ctg aat gag acc gtg gtt				1941
Thr Gly Trp Leu Glu Lys Asn Lys Asp Pro Leu Asn Glu Thr Val Val				

595	600	605	
gga ctg tac cag aag tct gca atg aaa act cta gct cag ctc ttc tct	1989		
Gly Leu Tyr Gln Lys Ser Ala Met Lys Thr Leu Ala Gln Leu Phe Ser			
610	615	620	625
gga gct caa act gct gaa gga gag gga gct ggc gga gga gcc aag aaa	2037		
Gly Ala Gln Thr Ala Glu Gly Glu Gly Ala Gly Gly Gly Ala Lys Lys			
630	635	640	
ggt ggt aag aag aag ggc tct tct ttc cag aca gta tct gcc att ttc	2085		
Gly Gly Lys Lys Lys Gly Ser Ser Phe Gln Thr Val Ser Ala Leu Phe			
645	650	655	
aga gag aat ttg aac aag ctg atg acc aac ctc agg agt acc cat oot	2133		
Arg Glu Asn Leu Asn Lys Leu Met Thr Asn Leu Arg Ser Thr His Pro			
660	665	670	
cac ttt gtg agg tgt atc atc gcc aat gag aca aaa act oot ggt gcc	2181		
His Phe Val Arg Cys Ile Ile Pro Asn Glu Thr Lys Thr Pro Gly Ala			
675	680	685	
atg gag cat gag att gtc ctc cac cag ctg agg tgt aac ggt gtg ctg	2229		
Met Glu His Glu Leu Val Leu His Gln Leu Arg Cys Asn Gly Val Leu			
690	695	700	705
gaa ggc atc cgc atc tgt agg aaa gga ttt cca agc aga atc att tat	2277		
Glu Gly Ile Arg Ile Cys Arg Lys Gly Phe Pro Ser Arg Ile Leu Tyr			
710	715	720	
gca gac ttc aaa cag aga tac aag gta tta aat gca agt gca atc oot	2325		
Ala Asp Phe Lys Gln Arg Tyr Lys Val Leu Asn Ala Ser Ala Ile Pro			
725	730	735	
gaa gga caa ttc att gat agc aag aag gcc tct gag aag ctc att gca	2373		
Glu Gly Gln Phe Ile Asp Ser Lys Lys Ala Ser Glu Lys Leu Leu Ala			

740	745	750	
too atc gac att gac cac acc cag tat aaa ttt gag cac acc aag gtc			2421
Ser Ile Asp Ile Asp His Thr Gln Tyr Lys Phe Gly His Thr Lys Val			
755	760	765	
ttt ttc aaa gct ggt ctt ctg gag ctc cta gag gag atg cga gat gac			2469
Phe Phe Lys Ala Gly Leu Leu Gly Leu Leu Glu Glu Met Arg Asp Asp			
770	775	780	785
aag ctg gcc cag ctg att acc cga acc cag gcc agg tgc aga gag ttc			2517
Lys Leu Ala Gln Leu Ile Thr Arg Thr Gln Ala Arg Cys Arg Gly Phe			
790	795	800	
ttg gca aga gtc gag tcc cag agg atg gtc gag aga agg gag gcc atc			2565
Leu Ala Arg Val Glu Tyr Gln Arg Met Val Glu Arg Arg Glu Ala Ile			
805	810	815	
tto tgt atc cag tcc aat atc aga tcc ttc atg aat gtc aag cac tag			2613
Phe Cys Ile Gln Tyr Asn Ile Arg Ser Phe Met Asn Val Lys His Trp			
820	825	830	
ccc tgg atg aaa ctc ttc ttc aag atc aag cct ctg ttg aag agt gca			2661
Pro Trp Met Lys Leu Phe Phe Lys Ile Lys Pro Leu Leu Lys Ser Ala			
835	840	845	
gaa act gag aag gag atg gcc acc atg aag gaa gaa ttt cag aaa att			2709
Glu Thr Glu Lys Glu Met Ala Thr Met Lys Glu Glu Phe Gln Lys Ile			
850	855	860	865
aaa gac gaa ctt gcc aag tcc gag gca aaa agg aag gaa ctg gaa gaa			2757
Lys Asp Glu Leu Ala Lys Ser Glu Ala Lys Arg Lys Glu Leu Glu Glu			
870	875	880	
aag atg gtc aag ctg ttg aaa gaa aaa aat gac ttg cag ctc caa gtt			2805
Lys Met Val Thr Leu Leu Lys Glu Lys Asn Asp Leu Gln Leu Gln Val			

885	890	895	
cag gct gaa gcc gaa ggc ttg gct gat gca gag gaa agg tgt gac cag			2853
Gln Ala Glu Ala Glu Gly Leu Ala Asp Ala Glu Glu Arg Cys Asp Gln			
900	905	910	
cta atc aaa acc aaa atc cag cta gaa gcc aaa atc aaa gag gtg act			2901
Leu Ile Lys Thr Lys Ile Gln Leu Glu Ala Lys Ile Lys Glu Val Thr			
915	920	925	
gag aga gct gag gat gag gaa gag atc aat gct gag ctg aca gcc aag			2949
Glu Arg Ala Glu Asp Glu Glu Glu Ile Asn Ala Glu Leu Thr Ala Lys			
930	935	940	945
aag agg aaa ctg gag gat gaa tgt tca gaa ctg aag aaa gac att gat			2997
Lys Arg Lys Leu Glu Asp Glu Cys Ser Glu Leu Lys Lys Asp Ile Asp			
950	955	960	
gac ctt gag ctg aca ctg gcc aag gtt gag aag gag aaa cat gcc aca			3045
Asp Leu Glu Leu Thr Leu Ala Lys Val Glu Lys Glu Lys His Ala Thr			
965	970	975	
gaa aac aag gtg aaa aac ctg aca gaa gag atg gca ggt ctg gat gaa			3093
Glu Asn Lys Val Lys Asn Leu Thr Glu Glu Met Ala Gly Leu Asp Glu			
980	985	990	
acc att gct aag ctg acc aag gag aag aag gct ctg cag gag gcc cac			3141
Thr Ile Ala Lys Leu Thr Lys Glu Lys Lys Ala Leu Gln Glu Ala His			
995	1000	1005	
cag cag acc ctg gat gac ctg cag gca gag gag gac aaa gtc aac			3189
Gln Gln Thr Leu Asp Asp Leu Gln Ala Glu Glu Asp Lys Val Asn			
1010	1015	1020	
acc ctg acc aaa gct aaa ctg aaa ctt gaa caa caa gta gat gat			3231
Thr Leu Thr Lys Ala Lys Ile Lys Leu Glu Gln Gln Val Asp Asp			

1025	1030	1035	
ctt gaa gag tcc ttg gag	caa gaa aag aaa ctt	cgc atg gac cta	3276
Leu Glu Gly Ser Leu Glu	Gln Glu Lys Lys Leu	Arg Met Asp Leu	
1040	1045	1050	
gaa agc gct aag agc aaa	ctt gag ggt gac ttg	aag ttg gcc caa	3321
Glu Arg Ala Lys Arg Lys	Leu Glu Gly Asp Leu	Lys Leu Ala Gln	
1055	1060	1065	
gaa tcc ata atg gac att	gaa aat gag aaa cag	caa ctt gat gaa	3366
Glu Ser Ile Met Asp Ile	Glu Asn Glu Lys Gln	Gln Leu Asp Glu	
1070	1075	1080	
aag ctc aaa aag aaa gag	ttt gaa atc agc aat	ctg caa agc aag	3411
Lys Leu Lys Lys Lys Glu	Phe Glu Ile Ser Asn	Leu Gln Ser Lys	
1085	1090	1095	
att gaa gat gaa cag gca	ctt ggc att caa ttg	cag aag aaa att	3456
Ile Glu Asp Glu Gln Ala	Leu Gly Ile Gln Leu	Gln Lys Lys Ile	
1100	1105	1110	
aaa gaa ttg caa gcc cgc	att gag gag ctg gag	gag gaa atc gag	3501
Lys Glu Leu Gln Ala Arg	Ile Glu Glu Leu Glu	Glu Glu Ile Glu	
1115	1120	1125	
gca gag cgg gcc tcc cgg	gcc aaa gca gag aag	cag cgc tct gac	3546
Ala Glu Arg Ala Ser Arg	Ala Lys Ala Glu Lys	Gln Arg Ser Asp	
1130	1135	1140	
ctc tcc cgg gag ctg gag	gag atc agc gag aag	ctg gaa gaa gcc	3591
Leu Ser Arg Glu Leu Glu	Glu Ile Ser Glu Arg	Leu Glu Glu Ala	
1145	1150	1155	
ggt gag gcc act tca gcc	cag att gag atg aac	aag aag cgg gag	3636
Gly Gly Ala Thr Ser Ala	Gln Ile Glu Met Asn	Lys Lys Arg Glu	

1160	1165	1170	
gct gag ttc cag aaa atg cgc agg gac ctg gag gag gcc acc cta			3681
Ala Glu Phe Gln Lys Met Arg Arg Asp Leu Glu Glu Ala Thr Leu			
1175	1180	1185	
cag cat gaa gcc aca ggc gcc acc ctg agg aag aag cat gca gat			3726
Gln His Glu Ala Thr Ala Ala Thr Leu Arg Lys Lys His Ala Asp			
1190	1195	1200	
agt gtg gcc gag ctt ggg gag cag att gac aac ctg cag cga gtg			3771
Ser Val Ala Glu Leu Gly Glu Gln Ile Asp Asn Leu Gln Arg Val			
1205	1210	1215	
aag cag aag ctg gag aag gag aag agt gag atg aag atg gag att			3816
Lys Gln Lys Leu Glu Lys Glu Lys Ser Glu Met Lys Met Glu Ile			
1220	1225	1230	
gat gac ctt gct agt aat gta gaa acg gtc tcc aac gcc aag gga			3861
Asp Asp Leu Ala Ser Asn Val Glu Thr Val Ser Lys Ala Lys Gly			
1235	1240	1245	
aac cta gag aaa atg tgc cgg act cta gag gac caa ctg agt gaa			3906
Asn Leu Glu Lys Met Cys Arg Thr Leu Glu Asp Gln Leu Ser Glu			
1250	1255	1260	
ctg aac tca aag gaa gag gag cag cag cgg ctg atc aat gac ctg			3951
Leu Lys Ser Lys Glu Glu Glu Gln Gln Arg Leu Ile Asn Asp Leu			
1265	1270	1275	
act gcc cag aag gag cgc ctg cag act gaa tct ggt gag tit tca			3996
Thr Ala Gln Arg Gly Arg Leu Gln Thr Glu Ser Gly Glu Phe Ser			
1280	1285	1290	
cgc cag ctt gat gaa aag gaa gct ctg atg tct cag tta tca aga			4041
Arg Gln Leu Asp Glu Lys Glu Ala Leu Val Ser Gln Leu Ser Arg			

1295	1300	1305	
ggc aag caa gcc ttt sct caa cag att gaa gaa tta aag agg caa			4086
Gly Lys Gln Ala Phe Thr Gln Gln Ile Glu Glu Leu Lys Arg Gln			
1310	1315	1320	
ott gaa gag gag ata aaa gcc aag aac gcc ctg gag cat gcc ctg			4131
Leu Glu Glu Glu Ile Lys Ala Lys Asn Ala Leu Ala His Ala Leu			
1325	1330	1335	
cag tat tcc cgc caa gac tgt gac ctg ctg cag gaa cag tat gag			4176
Gln Ser Ser Arg His Asp Cys Asp Leu Leu Arg Glu Gln Tyr Glu			
1340	1345	1350	
gag gag cag gaa tcc aag gcc gag ctg cag aga gca ctg tcc aag			4221
Glu Glu Gln Glu Ser Lys Ala Glu Leu Gln Arg Ala Leu Ser Lys			
1355	1360	1365	
gcc aac acc gag att gcc caa tgg agg acc aaa tac gag acc gac			4266
Ala Asn Thr Glu Val Ala Gln Trp Arg Thr Lys Tyr Glu Thr Asp			
1370	1375	1380	
gcc atc cag cgc aca gag gag ctg gag gag gcc aag aag aag ctg			4311
Ala Ile Gln Arg Thr Glu Glu Leu Glu Glu Ala Lys Lys Lys Leu			
1385	1390	1395	
gcc cag cgg ctg cag gca gct gag gaa cat gta gaa gct gtg aac			4356
Ala Gln Arg Leu Gln Ala Ala Glu Glu His Val Glu Ala Val Asn			
1400	1405	1410	
gcc aaa tgt gct tcc ctg gaa aag aag aag cag cgg ctg cag aat			4401
Ala Lys Cys Ala Ser Leu Glu Lys Thr Lys Gln Arg Leu Gln Asn			
1415	1420	1425	
gag gta gag gac ctg atg ott gat gta gag agg aca aat gcc gcc			4446
Glu Val Glu Asp Leu Met Leu Asp Val Glu Arg Thr Asn Ala Ala			

1430	1435	1440	
tgt gcc gcc ctt gac aaa aag caa agg aac ttc gat aag atc ctg			4491
Cys Ala Ala Leu Asp Lys Lys Gln Arg Asn Phe Asp Lys Ile Leu			
1445	1450	1455	
gca gaa tgg aaa cag aaa tgt gag gaa acg cat gct gag ctt gag			4526
Ala Glu Trp Lys Gln Lys Cys Glu Glu Thr His Ala Glu Leu Glu			
1460	1465	1470	
gcc tcc cag aag gag gcc cgt tcc ctt gcc act gag ctg ttc aag			4581
Ala Ser Gln Lys Glu Ala Arg Ser Leu Gly Thr Glu Leu Phe Lys			
1475	1480	1485	
ata aag aat gcc tat gag gaa tot ttg gat cag cta gaa acc ctg			4626
Ile Lys Asn Ala Tyr Glu Glu Ser Leu Asp Gln Leu Glu Thr Leu			
1490	1495	1500	
aag cga gag aac aaa aac tta cag cag gag att tct gac ctg aag			4671
Lys Arg Glu Asn Lys Asn Leu Gln Gln Glu Ile Ser Asp Leu Thr			
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gaa cag att gca gaa gga ggg aaa cgt atc cat gaa ctg gag aaa			4716
Glu Gln Ile Ala Glu Gly Gly Lys Arg Ile His Glu Leu Glu Lys			
1520	1525	1530	
ata aag aaa caa gtc gaa caa gaa aag tgt gaa ctt cag gct gct			4761
Ile Lys Lys Gln Val Glu Gln Glu Lys Cys Glu Leu Gln Ala Ala			
1535	1540	1545	
tta gaa gaa gca gag gca tot ctt gaa cat gaa gag gga aag atc			4806
Leu Glu Glu Ala Glu Ala Ser Leu Glu His Glu Glu Gly Lys Ile			
1550	1555	1560	
ctg cga atc cag ctt gag ttg aac caa gtc aag tot gag gtt gat			4851
Leu Arg Ile Gln Leu Glu Leu Asn Gln Val Lys Ser Glu Val Asp			

1565	1570	1575	
agg aag att gct gaa aag gat gag gaa att gac cag ctg aag aga			4896
Arg Lys Ile Ala Glu Lys Asp Glu Glu Ile Asp Gln Leu Lys Arg			
1580	1585	1590	
aac cac att aga atc gtg gag tcc atg cag agc aag ctg gat got			4941
Asn His Ile Arg Ile Val Glu Ser Met Gln Ser Thr Leu Asp Ala			
1595	1600	1605	
gag atc agg agt agg aat gat gcc att agg ctg aag aag aag atg			4986
Glu Ile Arg Ser Arg Asn Asp Ala Ile Arg Leu Lys Lys Lys Met			
1610	1615	1620	
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Glu Gly Asp Leu Asn Glu Met Glu Ile Gln Leu Asn His Ala Asn			
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cag atc gct gct gag gcc ctg agg aac tac agg aac acc gaa ggc			5076
Arg Met Ala Ala Glu Ala Leu Arg Asn Tyr Arg Asn Thr Gln Gly			
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atc ctg aag gat acc cag atc cac ctg gat gat gct ctg cgg agc			5121
Ile Leu Lys Asp Thr Gln Ile His Leu Asp Asp Ala Leu Arg Ser			
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cag gag gac ctg aag gaa cag ctg gcc atg gtg gag cgc aga gcc			5166
Gln Glu Asp Leu Lys Glu Gln Leu Ala Met Val Glu Arg Arg Ala			
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aac ctg ctg cag gct gag atc gag gag ctg cgg gcc aat ctg gaa			5211
Asn Leu Leu Gln Ala Glu Ile Glu Glu Leu Arg Ala Thr Leu Glu			
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Gln Thr Glu Arg Ser Arg Lys Ile Ala Glu Gln Glu Leu Leu Asp			

1700	1705	1710	
gac agt gag cgt gtt cag cta ctg cac acc cag aac acc agc ctg			5301
Ala Ser Glu Arg Val Gln Leu Leu His Thr Gln Asn Thr Ser Leu			
1715	1720	1725	
atc aac acc aag aag aag ctg gag aca gat att tcc caa atg caa			5346
Ile Asn Thr Lys Lys Lys Leu Glu Thr Asp Ile Ser Gln Met Gln			
1730	1735	1740	
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Gly Glu Met Glu Asp His Leu Gln Glu Ala Arg Asn Ala Glu Glu			
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Lys Ala Lys Lys Ala Ile Thr Asp Ala Ala Met Met Ala Glu Glu			
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ctg aag aag gag cag gac acc agc gcc cac ctg gag cag atg aag			5481
Leu Lys Lys Glu Gln Asp Thr Ser Ala His Leu Glu Arg Met Lys			
1775	1780	1785	
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Glu Ala Glu Gln Leu Ala Leu Lys Gly Gly Lys Lys Gln Ile Gln			
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Lys Leu Glu Ala Arg Val Arg Glu Leu Glu Gly Glu Val Glu Ser			
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Glu Gln Lys Arg Asn Ala Glu Ala Val Lys Gly Leu Arg Lys His			

1835	1840	1845	
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Glu Arg Arg Val Lys Glu Leu Thr Tyr Gln Thr Glu Glu Asp Arg			
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aag aat att ctc agg att caa gat ttg gta gat aaa ctt cag gca			5751
Lys Asn Ile Leu Arg Leu Gln Asp Leu Val Asp Lys Leu Gln Ala			
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aaa gtg aaa tct tat aag aga caa gct gag gag gct gag gaa caa			5796
Lys Val Lys Ser Tyr Lys Arg Gln Ala Glu Glu Ala Glu Glu Gln			
1880	1885	1890	
tcc aac acc aat cta gct aaa ttc cga aag ctc cag cat gag ctg			5841
Ser Asn Thr Asn Leu Ala Lys Phe Arg Lys Leu Gln His Glu Leu			
1895	1900	1905	
gag gag gcc gag gaa cgg gct gac att gct gag tcc cag gtg aac			5886
Glu Glu Ala Glu Glu Arg Ala Asp Ile Ala Glu Ser Gln Val Asn			
1910	1915	1920	
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Lys Leu Arg Val Lys Ser Arg Glu Val His Thr Lys Val Ile Ser			
1925	1930	1935	
gaa gag tga tcatgtctgt atgcatgga atgactgag aaggcaca			5980
Glu Glu			
1940			
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<213> Homo sapiens

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 35 40 45

Lys Gly Thr Ile Gln Ser Arg Glu Gly Gly Lys Val Thr Val Lys Thr
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Glu Gly Gly Ala Thr Leu Thr Val Lys Asp Asp Gln Val Phe Pro Met
 65 70 75 80

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 85 90 95

Leu His Glu Pro Ala Val Leu Tyr Asn Leu Lys Glu Arg Tyr Ala Ala
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 115 120 125

Tyr Lys Trp Leu Pro Val Tyr Lys Pro Glu Val Val Thr Ala Tyr Arg
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Gly Lys Lys Arg Gln Gly Ala Pro Pro His Ile Phe Ser Ile Ser Asp
145 150 155 160

Asn Ala Tyr Gln Phe Met Leu Thr Asp Arg Glu Asn Gln Ser Ile Leu
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180 185 190

Ile Gln Tyr Phe Ala Thr Ile Ala Val Thr Gly Glu Lys Lys Lys Glu
195 200 205

Glu Ile Thr Ser Gly Lys Ile Gln Gly Thr Leu Glu Asp Gln Ile Ile
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Ser Ala Asn Pro Leu Leu Glu Ala Phe Gly Asn Ala Lys Thr Val Arg
225 230 235 240

Asn Asp Asn Ser Ser Arg Phe Gly Lys Phe Ile Arg Ile His Phe Gly
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Thr Thr Gly Lys Leu Ala Ser Ala Asp Ile Glu Thr Tyr Leu Leu Glu
260 265 270

Lys Ser Arg Val Val Phe Gln Leu Lys Ala Glu Arg Ser Tyr His Ile
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Phe Tyr Gln Ile Thr Ser Asn Lys Lys Pro Glu Leu Ile Glu Met Leu
290 295 300

Leu Ile Thr Thr Asn Pro Tyr Asp Tyr Pro Phe Val Ser Gln Gly Glu
305 310 315 320

Ile Ser Val Ala Ser Ile Asp Asp Gln Glu Glu Leu Met Ala Thr Asp
325 330 335

Ser Ala Ile Asp Ile Leu Gly Phe Thr Asn Glu Glu Lys Val Ser Ile
340 345 350

Tyr Lys Leu Thr Gly Ala Val Met His Tyr Gly Asn Leu Lys Phe Lys
355 360 365

Gln Lys Gln Arg Glu Glu Gln Ala Glu Pro Asp Gly Thr Glu Val Ala
370 375 380

Asp Lys Ala Ala Tyr Leu Gln Ser Leu Asn Ser Ala Asp Leu Leu Lys
385 390 395 400

Ala Leu Cys Tyr Pro Arg Val Lys Val Gly Asn Glu Tyr Val Thr Lys
405 410 415

Gly Glu Thr Val Glu Glu Val Ser Asn Ala Val Gly Ala Leu Ala Lys
420 425 430

Ala Val Tyr Glu Lys Met Phe Leu Trp Met Val Ala Arg Ile Asn Gln
435 440 445

Gln Leu Asp Thr Lys Gln Pro Arg Gln Tyr Phe Ile Gly Val Leu Asp
450 455 460

Ile Ala Gly Phe Glu Ile Phe Asp Phe Asn Ser Leu Glu Gln Leu Cys
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Ile Asn Phe Thr Asn Glu Lys Leu Gln Gln Phe Phe Asn His His Met
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Gly Lys Ser Ala Asn Phe Gln Lys Pro Lys Val Val Lys Gly Lys Ala
565 570 575

Glu Ala His Phe Ala Leu Ile His Tyr Ala Gly Val Val Asp Tyr Asn
580 585 590

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595 600 605

Val Gly Leu Tyr Gln Lys Ser Ala Met Lys Thr Leu Ala Gln Leu Phe
610 615 620

Ser Gly Ala Gln Thr Ala Glu Gly Glu Gly Ala Gly Gly Gly Ala Lys
625 630 635 640

Lys Gly Gly Lys Lys Lys Gly Ser Ser Phe Gln Thr Val Ser Ala Leu
645 650 655

Phe Arg Glu Asn Leu Asn Lys Leu Met Thr Asn Leu Arg Ser Thr His
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Pro His Phe Val Arg Cys Ile Ile Pro Asn Glu Thr Lys Thr Pro Gly
675 680 685

Ala Met Glu His Glu Leu Val Leu His Gln Leu Arg Cys Asn Gly Val
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Leu Glu Gly Ile Arg Ile Cys Arg Lys Gly Phe Pro Ser Arg Ile Leu
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Tyr Ala Asp Phe Lys Gln Arg Tyr Lys Val Leu Asn Ala Ser Ala Ile
725 730 735

Pro Glu Gly Gln Phe Ile Asp Ser Lys Lys Ala Ser Glu Lys Leu Leu
740 745 750

Ala Ser Ile Asp Ile Asp His Thr Gln Tyr Lys Phe Gly His Thr Lys
755 760 765

Val Phe Phe Lys Ala Gly Leu Leu Gly Leu Leu Glu Glu Met Arg Asp
770 775 780

Asp Lys Leu Ala Gln Leu Ile Thr Arg Thr Gln Ala Arg Cys Arg Gly
785 790 795 800

Phe Leu Ala Arg Val Glu Tyr Gln Arg Met Val Glu Arg Arg Glu Ala
805 810 815

Ile Phe Cys Ile Gln Tyr Asn Ile Arg Ser Phe Met Asn Val Lys His
820 825 830

Tyr Pro Trp Met Lys Leu Phe Phe Lys Ile Lys Pro Leu Leu Lys Ser
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850

855

860

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865

870

875

880

Glu Lys Met Val Thr Leu Leu Lys Glu Lys Asn Asp Leu Gln Leu Gln

885

890

895

Val Gln Ala Glu Ala Glu Gly Leu Ala Asp Ala Glu Glu Arg Cys Asp

900

905

910

Gln Leu Ile Lys Thr Lys Ile Gln Leu Glu Ala Lys Ile Lys Glu Val

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920

925

Thr Glu Arg Ala Glu Asp Glu Glu Glu Ile Asn Ala Glu Leu Thr Ala

930

935

940

Lys Lys Arg Lys Leu Glu Asp Glu Cys Ser Glu Leu Lys Lys Asp Ile

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Asp Asp Leu Glu Leu Thr Leu Ala Lys Val Glu Lys Glu Lys His Ala

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985

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1805 1810 1815

Gln Lys Leu Glu Ala Arg Val Arg Glu Leu Glu Gly Glu Val Glu
1820 1825 1830

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1835 1840 1845

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Arg Lys Asn Ile Leu Arg Leu Gln Asp Leu Val Asp Lys Leu Gln
1865 1870 1875

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1895 1900 1905

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Lys Thr Ser Val Phe Val Val Asp Pro Lys Glu Ser Tyr Val Lys Ala

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Tyr Phe Ala Thr Ile Ala Val Thr Gly Glu Lys Lys Lys Glu Glu Pro
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 550 555 560

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 565 570 575

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 His Phe Ser Leu Val His Tyr Ala Gly Thr Val Asp Tyr Asn Ile Ala
 580 585 590

ggc tgg ctg gac aac aac aag gac ccc ctg aat gag aet gtg gtg ggg 1941
 Gly Trp Leu Asp Lys Asn Lys Asp Pro Leu Asn Glu Thr Val Val Gly
 595 600 605 610

ctg tac cag aag tct gca atg aag aet ctg gct ttc ctc ttc tct ggg 1989
 Leu Tyr Gln Lys Ser Ala Met Lys Thr Leu Ala Phe Leu Phe Ser Gly
 615 620 625

gca caa aet gct gaa gca gag gat gat gat gaa aag aac gat ggc aac 2037

Ala Gln Thr Ala Glu Ala Glu Gly Gly Gly Gly Lys Lys Gly Gly Lys	
630 635 640	
aag aag gag tct tct ttc cag aca gtc tca gct ctt ttc agg gag aat	2085
Lys Lys Gly Ser Ser Phe Gln Thr Val Ser Ala Leu Phe Arg Glu Asn	
645 650 655	
ttg aat aag ctg atg acc aac ttg agg agc act cac ccc ccc ttt gtc	2133
Leu Asn Lys Leu Met Thr Asn Leu Arg Ser Thr His Pro His Phe Val	
660 665 670	
cgg tgc atc atc ccc aat gaa act aaa act cct ggt gcc atg gag cat	2181
Arg Cys Ile Ile Pro Asn Glu Thr Lys Thr Pro Gly Ala Met Glu His	
675 680 685 690	
gag ctt gtc ctg cat cag ctg agg tgt aac ggt gtc ctg gaa ggc atc	2229
Glu Leu Val Leu His Gln Leu Arg Cys Asn Gly Val Leu Glu Gly Ile	
695 700 705	
ggc atc tgc agg aaa ggc ttc cca agc aga atc ctt tat gca gac ttc	2277
Arg Ile Cys Arg Lys Gly Phe Pro Ser Arg Ile Leu Tyr Ala Asp Phe	
710 715 720	
aaa cag aga tac aag gtt cta aat ggc agt gct atc cca gag ggt cag	2325
Lys Gln Arg Tyr Lys Val Leu Asn Ala Ser Ala Ile Pro Glu Gly Gln	
725 730 735	
ttc att gac agc aag aag gct tct gag aaa ctt ctc ggc tct att gaa	2373
Phe Ile Asp Ser Lys Lys Ala Ser Glu Lys Leu Leu Gly Ser Ile Glu	
740 745 750	
att gac caa acc cag tac aaa ttc ggt cat acc aag gtt ttc ttc aaa	2421
Ile Asp His Thr Gln Tyr Lys Phe Gly His Thr Lys Val Phe Phe Lys	
755 760 765 770	
gct ggc ctg ctg gga act cta gaa gaa atg cga gat gaa aag cta gct	2469

Ala Gly Leu Leu Gly Thr Leu Glu Glu Met Arg Asp Glu Lys Leu Ala
 775 780 785

cca ctc atc acg cgc act cca gcc ata tgc agg gag ttc ctg atg aga 2517
 Gln Leu Ile Thr Arg Thr Gln Ala Ile Cys Arg Gly Phe Leu Met Arg
 790 795 800

atg gag ttc aga aag atg atg gag agg aga gag tcc atc ttc tgc att 2565
 Val Glu Phe Arg Lys Met Met Glu Arg Arg Glu Ser Ile Phe Cys Ile
 805 810 815

cag tac aac atc cgt gct ttc atg aat gtc aag ccc tgg ccc tgg atg 2613
 Gln Tyr Asn Ile Arg Ala Phe Met Asn Val Lys His Trp Pro Trp Met
 820 825 830

aag ctg tat ttc aag atc aag ccc ctc ctc aag agt gca gag aca gag 2661
 Lys Leu Tyr Phe Lys Ile Lys Pro Leu Leu Lys Ser Ala Glu Thr Glu
 835 840 845 850

aag gag atg gcc aac atg aag gaa gaa ttt gag aaa acc aac gaa gag 2709
 Lys Glu Met Ala Asn Met Lys Glu Glu Phe Glu Lys Thr Lys Glu Glu
 855 860 865

ctg gct aag aca gag gca aaa agg aaa gaa cta gaa gaa aag atg gtc 2757
 Leu Ala Lys Thr Glu Ala Lys Arg Lys Glu Leu Glu Glu Lys Met Val
 870 875 880

aag ata atg cca gag aaa aat gac tta cca ctc cca gtt cca gct gaa 2805
 Thr Leu Met Gln Glu Lys Asn Asp Leu Gln Leu Gln Val Gln Ala Glu
 885 890 895

gca gat gcc tgg gct gat gca gag gaa aga tgt gat cag ttg att aaa 2853
 Ala Asp Ala Leu Ala Asp Ala Glu Glu Arg Cys Asp Gln Leu Ile Lys
 900 905 910

acc aaa atc caa ctt gag gcc aac atc aaa gag gta act gaa aga gct 2901

Thr Lys Ile Gln Leu Glu Ala Lys Ile Lys Glu Val Thr Glu Arg Ala	
915	920 925 930
gag gat gag gaa gag aac aat gct gag ctg aca gcc aag aag agg aaa	2949
Glu Asp Glu Glu Glu Ile Asn Ala Glu Leu Thr Ala Lys Lys Arg Lys	
935	940 945
ctg gag gat gaa tgt tca gag ctg aag aaa gcc att gat gac att gag	2997
Leu Glu Asp Glu Cys Ser Glu Leu Lys Lys Asp Ile Asp Asp Leu Glu	
950	955 960
ctg aca ctg gcc aag gtt gag aag gag aaa cat gcc aca gag aac aag	3045
Leu Thr Leu Ala Lys Val Glu Lys Glu Lys His Ala Thr Glu Asn Lys	
965	970 975
ctg aaa aac ctg aca gaa gag atg gaa ggt ctg gat gaa aac att gct	3093
Val Lys Asn Leu Thr Glu Glu Met Ala Gly Leu Asp Glu Thr Ile Ala	
980	985 990
aag ctg aca aag gag aag aag gct ctg cag gag gcc cac cag cag	3138
Lys Leu Thr Lys Glu Lys Lys Ala Leu Gln Glu Ala His Gln Gln	
995	1000 1005
acc ctg gat gac ctg cag atg gag gag gac aaa gtc aac acc ctg	3183
Thr Leu Asp Asp Leu Gln Met Glu Glu Asp Lys Val Asn Thr Leu	
1010	1015 1020
acc aaa gct aaa acc aag cta gaa cag caa gtg gac gat att gaa	3228
Thr Lys Ala Lys Thr Lys Leu Glu Gln Gln Val Asp Asp Leu Glu	
1025	1030 1035
gga tct ctg gaa caa gaa aag aaa att tgc atg gac tta gaa aga	3273
Gly Ser Leu Glu Gln Glu Lys Lys Leu Cys Met Asp Leu Glu Arg	
1040	1045 1050
gcc aag aga aaa ctg gag ggt gac cta aaa tgg gcc caa gaa taa	3318

Ala Lys Arg Lys Leu Glu Gly Asp Leu Lys Leu Ala Gln Glu Ser	
1055	1060 1065
aca atg gat acc gaa aat gac aaa cag caa ctt aat gag aaa ctc	3363
Thr Met Asp Thr Glu Asn Asp Lys Gln Gln Leu Asn Glu Lys Leu	
1070	1075 1080
aaa aag aaa gag ttt gaa atg agc aat ctg caa gcc aag att gaa	3408
Lys Lys Lys Glu Phe Glu Met Ser Asn Leu Gln Gly Lys Ile Glu	
1085	1090 1095
gat gaa caa gcc ctt gca atg cag cta caa aag aag atc aaa gaa	3453
Asp Glu Gln Ala Leu Ala Met Gln Leu Gln Lys Lys Ile Lys Glu	
1100	1105 1110
tta cag gcc cgc att gag gag ctg gag gag gaa atc gag gca gag	3498
Leu Gln Ala Arg Ile Glu Glu Leu Glu Glu Glu Ile Glu Ala Glu	
1115	1120 1125
agg gcc tcc cgg gcc aaa gca gaa aag cag cgc tct gac ctc tcc	3543
Arg Ala Ser Arg Ala Lys Ala Glu Lys Gln Arg Ser Asp Leu Ser	
1130	1135 1140
cgg gag ctg gag gag atc agt gag agc ctg gaa gaa gcc gat gag	3588
Arg Glu Leu Glu Glu Ile Ser Glu Arg Leu Glu Glu Ala Gly Gly	
1145	1150 1155
gcc act tca gcc cag att gag ttg aac aag aag cgg gag gct gag	3633
Ala Thr Ser Ala Gln Ile Glu Leu Asn Lys Lys Arg Glu Ala Glu	
1160	1165 1170
ttc cag aaa atg cgc agg gac ctg gaa gag tcc acc ctg cag cac	3678
Phe Gln Lys Met Arg Arg Asp Leu Glu Glu Ser Thr Leu Gln His	
1175	1180 1185
gaa gcc aag gca gct gct ctt cgg aag aag cac gca gat agt gtg	3723

Glu	Ala	Thr	Ala	Ala	Ala	Leu	Arg	Lys	Lys	His	Ala	Asp	Ser	Val	
1190						1195					1200				
gct	gag	ott	ggg	aag	cag	atc	gac	agc	ctt	cag	agg	gtc	aag	cag	3768
Ala	Glu	Leu	Gly	Lys	Gln	Ile	Asp	Ser	Leu	Gln	Arg	Val	Lys	Gln	
1205						1210					1215				
aag	ctg	gag	aag	gag	aag	agt	gag	ctg	aag	atg	gag	atc	aat	gac	3813
Lys	Leu	Glu	Lys	Glu	Lys	Ser	Glu	Leu	Lys	Met	Glu	Ile	Asn	Asp	
1220						1225					1230				
ctt	gct	agt	aac	atg	gag	act	gtc	tcc	aaa	gac	aag	gca	aac	ttt	3858
Leu	Ala	Ser	Asn	Met	Glu	Thr	Val	Ser	Lys	Ala	Lys	Ala	Asn	Phe	
1235						1240					1245				
gag	aaa	atg	tgc	cgc	acc	cta	gag	gac	cag	ctt	agt	gaa	ata	aaa	3903
Glu	Lys	Met	Cys	Arg	Thr	Leu	Glu	Asp	Gln	Leu	Ser	Glu	Ile	Lys	
1250						1255					1260				
aca	aag	gaa	gaa	gag	cag	caa	cgc	tta	ata	aat	gag	tta	tca	gac	3948
Thr	Lys	Glu	Glu	Glu	Gln	Gln	Arg	Leu	Ile	Asn	Glu	Leu	Ser	Ala	
1265						1270					1275				
cag	aag	gca	cgt	tta	caa	aca	gaa	tca	ggt	gag	ttt	tca	cga	cag	3993
Gln	Lys	Ala	Arg	Leu	His	Thr	Glu	Ser	Gly	Glu	Phe	Ser	Arg	Gln	
1280						1285					1290				
cta	gat	gaa	aaa	gat	gct	atg	gtt	tct	cag	cta	tcc	oga	ggc	aaa	4038
Leu	Asp	Glu	Lys	Asp	Ala	Met	Val	Ser	Gln	Leu	Ser	Arg	Gly	Lys	
1295						1300					1305				
caa	gca	ttt	aca	caa	cag	att	gaa	gaa	tta	aag	agg	cag	cta	gaa	4083
Gln	Ala	Phe	Thr	Gln	Gln	Ile	Glu	Glu	Leu	Lys	Arg	Gln	Leu	Glu	
1310						1315					1320				
gag	gag	act	aag	gac	aag	agc	act	ctg	gac	cat	gac	ctg	cag	tca	4128

Glu	Glu	Thr	Lys	Ala	Lys	Ser	Thr	Leu	Ala	His	Ala	Leu	Gln	Ser	
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Ala	Arg	His	Asp	Cys	Asp	Leu	Leu	Arg	Glu	Gln	Tyr	Glu	Glu	Glu	
1340															
cag	gaa	gac	aag	gct	gag	ctg	cag	agg	gaa	atg	taa	agg	gac	aac	4218
Gln	Glu	Ala	Lys	Ala	Glu	Leu	Gln	Arg	Gly	Met	Ser	Lys	Ala	Asn	
1355															
agt	gag	gtt	gac	cag	tgg	agg	acc	aag	tac	gag	acc	gac	gac	etc	4263
Ser	Glu	Val	Ala	Gln	Trp	Arg	Thr	Lys	Tyr	Glu	Thr	Asp	Ala	Ile	
1370															
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Gln	Arg	Thr	Glu	Glu	Leu	Glu	Glu	Ala	Lys	Lys	Lys	Leu	Ala	Gln	
1385															
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Arg	Leu	Gln	Asp	Ala	Glu	Glu	His	Val	Glu	Ala	Val	Asn	Ser	Lys	
1400															
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Cys	Ala	Ser	Leu	Glu	Lys	Thr	Lys	Gln	Arg	Leu	Gln	Asn	Glu	Val	
1415															
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Glu	Asp	Leu	Met	Ile	Asp	Val	Glu	Arg	Ser	Asn	Ala	Ala	Cys	Ile	
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Ala	Leu	Asp	Lys	Lys	Gln	Arg	Asn	Phe	Asp	Lys	Val	Leu	Ala	Glu	
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Trp	Lys	Gln	Lys	Tyr	Glu	Glu	Thr	Gln	Ala	Glu	Leu	Glu	Ala	Ser		
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Gln	Lys	Glu	Ser	Arg	Ser	Leu	Ser	Thr	Glu	Leu	Phe	Lys	Val	Lys		
1475						1480					1485					
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Asn	Ala	Tyr	Glu	Glu	Ser	Leu	Asp	His	Leu	Glu	Thr	Leu	Lys	Arg		
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Glu	Asn	Lys	Asn	Leu	Gln	Gln	Glu	Ile	Ser	Asp	Leu	Thr	Glu	Gln		
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Ile	Ala	Glu	Gly	Gly	Lys	His	Ile	His	Glu	Leu	Glu	Lys	Val	Lys		
1520						1525					1530					
aaa	caa	ctt	gat	cat	gag	aag	agt	gaa	cta	cag	act	tcc	cta	gag	4758	
Lys	Gln	Leu	Asp	His	Glu	Lys	Ser	Glu	Leu	Gln	Thr	Ser	Leu	Glu		
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Glu	Ala	Glu	Ala	Ser	Leu	Glu	His	Glu	Glu	Gly	Lys	Ile	Leu	Arg		
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Ile	Gln	Leu	Glu	Leu	Asn	Gln	Val	Lys	Ser	Glu	Ile	Asp	Arg	Lys		
1565						1570					1575					
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Ile	Ala	Glu	Lys	Asp	Glu	Glu	Leu	Asp	Gln	Leu	Lys	Arg	Asn	His		
1580						1585					1590					
ctc	aga	gtt	gtg	gag	tca	atg	cag	agt	aca	ctg	gat	gct	gag	atc	4938	

Leu Arg Val Val Glu Ser Met Gln Ser Thr Leu Asp Ala Glu Ile
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agg agc aga aat gat gct ctg agg atc aag aag aag atg gag gga 4983
 Arg Ser Arg Asn Asp Ala Leu Arg Ile Lys Lys Lys Met Glu Gly
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 Asp Leu Asn Glu Met Glu Ile Gln Leu Asn His Ala Asn Arg Gln
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 Ala Ala Glu Ala Leu Arg Asn Leu Arg Asn Thr Gln Gly Ile Leu
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 Lys Asp Thr Gln Leu His Leu Asp Asp Ala Ile Arg Gly Gln Asp
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 Asp Leu Lys Glu Gln Leu Ala Met Val Glu Arg Arg Ala Asn Leu
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 Met Gln Ala Glu Val Glu Glu Leu Arg Ala Ser Leu Glu Arg Thr
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Thr Lys Lys Lys Leu Glu Thr Asp Ile Ser Gln Ile Gln Gly Glu
 1730 1735 1740

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 Met Glu Asp Ile Val Gln Glu Ala Arg Asn Ala Glu Glu Lys Ala
 1745 1750 1755

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 Lys Lys Ala Ile Thr Asp Ala Ala Met Met Ala Glu Glu Leu Lys
 1760 1765 1770

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 Lys Glu Gln Asp Thr Ser Ala His Leu Glu Arg Met Lys Lys Asn
 1775 1780 1785

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 Met Glu Gln Thr Val Lys Asp Leu Gln Leu Arg Leu Gly Glu Ala
 1790 1795 1800

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 Glu Gln Leu Ala Leu Lys Gly Gly Lys Lys Gln Ile Gln Lys Leu
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 Lys His Asn Val Glu Ala Val Lys Gly Leu Arg Lys His Glu Arg
 1835 1840 1845

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 Arg Val Lys Glu Leu Thr Tyr Gln Thr Glu Glu Asp Arg Lys Asn
 1850 1855 1860

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Ile Leu Arg Leu Gln Asp Leu Val Asp Lys Leu Gln Thr Lys Val
 1865 1870 1875

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 Lys Ala Tyr Lys Arg Gln Ala Glu Glu Ala Glu Glu Gln Ser Asn
 1880 1885 1890

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 Val Asn Leu Ala Lys Phe Arg Lys Leu Gln His Glu Leu Glu Glu
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 Ala Glu Glu Arg Ala Asp Ile Ala Glu Ser Gln Val Asn Lys Leu
 1910 1915 1920

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Lys Ala Ile Val Gln Ser Arg Glu Gly Gly Lys Val Thr Ala Lys Thr		
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Glu Ala Gly Ala Thr Val Thr Val Lys Glu Asp Gln Val Phe Ser Met		
65	70	75
80		
Asn Pro Pro Lys Tyr Asp Lys Ile Glu Asp Met Ala Met Met Thr His		
85	90	95
Leu His Glu Pro Ala Val Leu Tyr Asn Leu Lys Glu Arg Tyr Ala Ala		
100	105	110
Trp Met Ile Tyr Thr Tyr Ser Gly Leu Phe Cys Val Thr Val Asn Pro		
115	120	125
Tyr Lys Trp Leu Pro Val Tyr Asn Pro Glu Val Val Thr Ala Tyr Arg		
130	135	140
Gly Lys Lys Arg Gln Glu Ala Pro Pro His Ile Phe Ser Ile Ser Asp		
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Asn Ala Tyr Gln Phe Met Leu Thr Asp Arg Glu Asn Gln Ser Ile Leu		

165

170

175

Ile Thr Gly Glu Ser Gly Ala Gly Lys Thr Val Asn Thr Lys Arg Val

180

185

190

Ile Glu Tyr Phe Ala Thr Ile Ala Val Thr Gly Glu Lys Lys Lys Glu

195

200

205

Glu Pro Ala Ser Gly Lys Met Gln Gly Thr Leu Glu Asp Gln Ile Ile

210

215

220

Ser Ala Asn Pro Leu Leu Glu Ala Phe Gly Asn Ala Lys Thr Val Arg

225

230

235

240

Asn Asp Asn Ser Ser Arg Phe Gly Lys Phe Ile Arg Ile His Phe Gly

245

250

255

Ala Thr Gly Lys Leu Ala Ser Ala Asp Ile Glu Thr Tyr Leu Leu Glu

260

265

270

Lys Ser Arg Val Thr Phe Gln Leu Lys Ala Glu Arg Ser Tyr His Ile

275

280

285

Phe Tyr Gln Ile Leu Ser Asn Lys Lys Pro Glu Leu Ile Glu Met Leu

290

295

300

Leu Ile Thr Thr Asn Pro Tyr Asp Phe Ala Phe Val Ser Gln Gly Glu

305 310 315 320

Ile Thr Val Pro Ser Ile Asp Asp Gln Glu Glu Leu Met Ala Thr Asp
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Ser Ala Val Asp Ile Leu Gly Phe Thr Ala Asp Glu Lys Val Ala Ile
340 345 350

Tyr Lys Leu Thr Gly Ala Val Met His Tyr Gly Asn Met Lys Phe Lys
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Gln Lys Gln Arg Glu Glu Gln Ala Glu Pro Asp Gly Thr Glu Val Ala
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Asp Lys Ala Ala Tyr Leu Thr Ser Leu Asn Ser Ala Asp Leu Leu Lys
385 390 395 400

Ser Leu Cys Tyr Pro Arg Val Lys Val Gly Asn Glu Phe Val Thr Lys
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Gly Gln Thr Val Gln Gln Val Tyr Asn Ala Val Gly Ala Leu Ala Lys
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Ala Ile Tyr Glu Lys Met Phe Leu Trp Met Val Thr Arg Ile Asn Gln
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Gln Leu Asp Thr Lys Gln Pro Arg Gln Tyr Phe Ile Gly Val Leu Asp

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Ile Ala Gly Phe Glu Ile Phe Asp Phe Asn Ser Leu Glu Gln Leu Cys

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Ile Asn Phe Thr Asn Glu Lys Leu Gln Gln Phe Phe Asn His His Met

485

490

495

Phe Val Leu Glu Gln Glu Glu Tyr Lys Lys Glu Gly Ile Glu Trp Glu

500

505

510

Phe Ile Asp Phe Gly Met Asp Leu Ala Ala Cys Ile Glu Leu Ile Glu

515

520

525

Lys Pro Met Gly Ile Phe Ser Ile Leu Glu Glu Glu Cys Met Phe Pro

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Lys Ala Thr Asp Thr Ser Phe Lys Asn Lys Leu Tyr Glu Gln His Leu

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555

560

Gly Lys Ser Asn Asn Phe Gln Lys Pro Lys Pro Ala Lys Gly Lys Pro

565

570

575

Glu Ala His Phe Ser Leu Val His Tyr Ala Gly Thr Val Asp Tyr Asn

580

585

590

Ile Ala Gly Trp Leu Asp Lys Asn Lys Asp Pro Leu Asn Glu Thr Val

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Val Gly Leu Tyr Gln Lys Ser Ala Met Lys Thr Leu Ala Phe Leu Phe

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Ser Gly Ala Gln Thr Ala Glu Ala Glu Gly Gly Gly Gly Lys Lys Gly

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Gly Gln Phe Ile Asp Ser Lys Lys Ala Ser Glu Lys Leu Leu Gly Ser

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Ile Glu Ile Asp His Thr Gln Tyr Lys Phe Gly His Thr Lys Val Phe

755

760

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Phe Lys Ala Gly Leu Leu Gly Thr Leu Glu Glu Met Arg Asp Glu Lys

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810

815

Cys Ile Glu Tyr Asn Ile Arg Ala Phe Met Asn Val Lys His Trp Pro

820

825

830

Trp Met Lys Leu Tyr Phe Lys Ile Lys Pro Leu Leu Lys Ser Ala Glu

835

840

845

Thr Glu Lys Glu Met Ala Asn Met Lys Glu Glu Phe Glu Lys Thr Lys

850

855

860

Glu Glu Leu Ala Lys Thr Glu Ala Lys Arg Lys Glu Leu Glu Glu Lys

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870

875

880

Met Val Thr Leu Met Gln Glu Lys Asn Asp Leu Gln Leu Gln Val Gln

885

890

895

Ala Glu Ala Asp Ala Leu Ala Asp Ala Glu Glu Arg Cys Asp Gln Leu

900

905

910

Ile Lys Thr Lys Ile Gln Leu Glu Ala Lys Ile Lys Glu Val Thr Glu

915

920

925

Arg Ala Glu Asp Glu Glu Glu Ile Asn Ala Glu Leu Thr Ala Lys Lys

930

935

940

Arg Lys Leu Glu Asp Glu Cys Ser Glu Leu Lys Lys Asp Ile Asp Asp

945

950

955

960

Leu Glu Leu Thr Leu Ala Lys Val Glu Lys Glu Lys His Ala Thr Glu

965

970

975

Asn Lys Val Lys Asn Leu Thr Glu Glu Met Ala Gly Leu Asp Glu Thr

980

985

990

Ile Ala Lys Leu Thr Lys Glu Lys Lys Ala Leu Gln Glu Ala His Gln

995

1000

1005

Gln Thr Leu Asp Asp Leu Gln Met Glu Glu Asp Lys Val Asn Thr

1010

1015

1020

Leu Thr Lys Ala Lys Thr Lys Leu Glu Gln Gln Val Asp Asp Leu

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1030

1035

Glu Gly Ser Leu Glu Gln Glu Lys Lys Leu Cys Met Asp Leu Glu

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1045

1050

Arg Ala Lys Arg Lys Leu Glu Gly Asp Leu Lys Leu Ala Gln Glu

1055

1060

1065

Ser Thr Met Asp Thr Glu Asn Asp Lys Gln Gln Leu Asn Glu Lys

1070

1075

1080

Leu Lys Lys Lys Glu Phe Glu Met Ser Asn Leu Gln Gly Lys Ile

1085

1090

1095

Glu Asp Glu Gln Ala Leu Ala Met Gln Leu Gln Lys Lys Ile Lys

1100

1105

1110

Glu Leu Gln Ala Arg Ile Glu Glu Leu Glu Glu Glu Ile Glu Ala

1115

1120

1125

Glu Arg Ala Ser Arg Ala Lys Ala Glu Lys Gln Arg Ser Asp Leu

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1135

1140

Ser Arg Glu Leu Glu Glu Ile Ser Glu Arg Leu Glu Glu Ala Gly

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1150

1155

Gly Ala Thr Ser Ala Gln Ile Glu Leu Asn Lys Lys Arg Glu Ala

1160

1165

1170

Glu Phe Gln Lys Met Arg Arg Asp Leu Glu Glu Ser Thr Leu Gln

1175

1180

1185

His Glu Ala Thr Ala Ala Ala Leu Arg Lys Lys His Ala Asp Ser

1190

1195

1200

Val Ala Glu Leu Gly Lys Gln Ile Asp Ser Leu Gln Arg Val Lys

1205

1210

1215

Gln Lys Leu Glu Lys Glu Lys Ser Glu Leu Lys Met Glu Ile Asn

1220

1225

1230

Asp Leu Ala Ser Asn Met Glu Thr Val Ser Lys Ala Lys Ala Asn

1235

1240

1245

Phe Glu Lys Met Cys Arg Thr Leu Glu Asp Gln Leu Ser Glu Ile

1250

1255

1260

Lys Thr Lys Glu Glu Glu Gln Gln Arg Leu Ile Asn Glu Leu Ser

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1270

1275

Ala Gln Lys Ala Arg Leu His Thr Glu Ser Gly Glu Phe Ser Arg

1280

1285

1290

Gln Leu Asp Glu Lys Asp Ala Met Val Ser Gln Leu Ser Arg Gly

1295

1300

1305

Lys Gln Ala Phe Thr Gln Gln Ile Glu Glu Leu Lys Arg Gln Leu

1310

1315

1320

Glu Glu Glu Thr Lys Ala Lys Ser Thr Leu Ala His Ala Leu Gln

1325

1330

1335

Ser Ala Arg His Asp Cys Asp Leu Leu Arg Glu Gln Tyr Glu Glu

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1345

1350

Glu Gln Glu Ala Lys Ala Glu Leu Gln Arg Gly Met Ser Lys Ala

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1360

1365

Asn Ser Glu Val Ala Gln Trp Arg Thr Lys Tyr Glu Thr Asp Ala

1370

1375

1380

Ile Gln Arg Thr Glu Glu Leu Glu Glu Ala Lys Lys Lys Leu Ala

1385

1390

1395

Gln Arg Leu Gln Asp Ala Glu Glu His Val Glu Ala Val Asn Ser

1400

1405

1410

Lys Cys Ala Ser Leu Glu Lys Thr Lys Gln Arg Leu Gln Asn Glu

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1420

1425

Val Glu Asp Leu Met Ile Asp Val Glu Arg Ser Asn Ala Ala Cys

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Ile Ala Leu Asp Lys Lys Gln Arg Asn Phe Asp Lys Val Leu Ala		
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Glu Trp Lys Gln Lys Tyr Glu Glu Thr Gln Ala Glu Leu Glu Ala		
1460	1465	1470
Ser Gln Lys Glu Ser Arg Ser Leu Ser Thr Glu Leu Phe Lys Val		
1475	1480	1485
Lys Asn Ala Tyr Glu Glu Ser Leu Asp His Leu Glu Thr Leu Lys		
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Arg Glu Asn Lys Asn Leu Gln Gln Glu Ile Ser Asp Leu Thr Glu		
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Gln Ile Ala Glu Gly Gly Lys His Ile His Glu Leu Glu Lys Val		
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Lys Lys Gln Leu Asp His Glu Lys Ser Glu Leu Gln Thr Ser Leu		
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Glu Glu Ala Glu Ala Ser Leu Glu His Glu Glu Gly Lys Ile Leu		
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Arg Ile Gln Leu Glu Leu Asn Gln Val Lys Ser Glu Ile Asp Arg		

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1570

1575

Lys Ile Ala Glu Lys Asp Glu Glu Leu Asp Gln Leu Lys Arg Asn

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1585

1590

His Leu Arg Val Val Glu Ser Met Gln Ser Thr Leu Asp Ala Glu

1595

1600

1605

Ile Arg Ser Arg Asn Asp Ala Leu Arg Ile Lys Lys Lys Met Glu

1610

1615

1620

Gly Asp Leu Asn Glu Met Glu Ile Gln Leu Asn His Ala Asn Arg

1625

1630

1635

Gln Ala Ala Glu Ala Leu Arg Asn Leu Arg Asn Thr Gln Gly Ile

1640

1645

1650

Leu Lys Asp Thr Gln Leu His Leu Asp Asp Ala Ile Arg Gly Gln

1655

1660

1665

Asp Asp Leu Lys Glu Gln Leu Ala Met Val Glu Arg Arg Ala Asn

1670

1675

1680

Leu Met Gln Ala Glu Val Glu Glu Leu Arg Ala Ser Leu Glu Arg

1685

1690

1695

Thr Glu Arg Gly Arg Lys Met Ala Glu Gln Glu Leu Leu Asp Ala

1700

1705

1710

Ser Glu Arg Val Gln Leu Leu His Thr Gln Asn Thr Ser Leu Ile

1715

1720

1725

Asn Thr Lys Lys Lys Leu Glu Thr Asp Ile Ser Gln Ile Gln Gly

1730

1735

1740

Glu Met Glu Asp Ile Val Gln Glu Ala Arg Asn Ala Glu Glu Lys

1745

1750

1755

Ala Lys Lys Ala Ile Thr Asp Ala Ala Met Met Ala Glu Glu Leu

1760

1765

1770

Lys Lys Glu Gln Asp Thr Ser Ala His Leu Glu Arg Met Lys Lys

1775

1780

1785

Asn Met Glu Gln Thr Val Lys Asp Leu Gln Leu Arg Leu Gly Glu

1790

1795

1800

Ala Glu Gln Leu Ala Leu Lys Gly Gly Lys Lys Gln Ile Gln Lys

1805

1810

1815

Leu Glu Ala Arg Val Arg Glu Leu Glu Ser Glu Val Glu Ser Glu

1820

1825

1830

Gln Lys His Asn Val Glu Ala Val Lys Gly Leu Arg Lys His Glu

1835

1840

1845

Arg Arg Val Lys Glu Leu Thr Tyr Gln Thr Glu Glu Asp Arg Lys

1850

1855

1860

Asn Ile Leu Arg Leu Gln Asp Leu Val Asp Lys Leu Gln Thr Lys

1865

1870

1875

Val Lys Ala Tyr Lys Arg Gln Ala Glu Glu Ala Glu Glu Gln Ser

1880

1885

1890

Asn Val Asn Leu Ala Lys Phe Arg Lys Leu Gln His Glu Leu Glu

1895

1900

1905

Glu Ala Glu Glu Arg Ala Asp Ile Ala Glu Ser Gln Val Asn Lys

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1915

1920

Leu Arg Val Lys Ser Arg Glu Val His Thr Lys Val Ile Ser Glu

1925

1930

1935

Glu

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1		5						10						15		

ctc	cga	aag	tct	gaa	agg	gag	cga	att	gaa	goc	cag	aac	aag	cct	ttt	96
Leu	Arg	Lys	Ser	Glu	Arg	Glu	Arg	Ile	Glu	Ala	Gln	Asn	Lys	Pro	Phe	
		20						25						30		

gat	gac	aag	aca	tca	gtc	ttt	gtg	gtg	gac	cct	aag	gag	tac	ttt	gtg	144
Asp	Ala	Lys	Thr	Ser	Val	Phe	Val	Val	Asp	Pro	Lys	Glu	Ser	Phe	Val	
		35						40						45		

aaa	gca	aca	gtg	cag	agg	agg	gaa	agg	ggg	aag	gtg	aca	gct	aag	acc	192
Lys	Ala	Thr	Val	Gln	Ser	Arg	Glu	Gly	Gly	Lys	Val	Thr	Ala	Lys	Thr	
		50						55						60		

aaa	gct	gga	gct	act	gta	aca	gtg	aaa	gat	gac	caa	gtc	ttc	ccc	atg	240
Glu	Ala	Gly	Ala	Thr	Val	Thr	Val	Lys	Asp	Asp	Gln	Val	Phe	Pro	Met	
65								70						75		80

aac	cct	ccc	aaa	tat	gac	aag	atc	gag	gac	atg	gcc	atg	atg	act	cat	288
Asn	Pro	Pro	Lys	Tyr	Asp	Lys	Ile	Glu	Asp	Met	Ala	Met	Met	Thr	His	
								85						90		95

cta	caa	gag	cct	gct	gtg	ctg	tac	aac	ctc	aaa	gag	cgc	tac	gaa	gcc	336
Leu	His	Glu	Pro	Ala	Val	Leu	Tyr	Asn	Leu	Lys	Glu	Arg	Tyr	Ala	Ala	
								100						105		110

tgg	atg	atc	tac	acc	tac	tca	ggc	ttg	ttc	tgt	gtc	act	gtc	aac	ccc	384
Trp	Met	Ile	Tyr	Thr	Tyr	Ser	Gly	Leu	Phe	Cys	Val	Thr	Val	Asn	Pro	

115	120	125	
taa aag tag ttg cca gtg tat aat gca gaa gtg gtg aca ggc tac cga			432
Tyr Lys Trp Leu Pro Val Tyr Asn Ala Glu Val Val Thr Ala Tyr Arg			
130	135	140	
ggc aaa aag cgc cag gaa ggc cca ccc ccc atc ttc tcc atc tct gac			480
Gly Lys Lys Arg Gln Glu Ala Pro Pro His Ile Phe Ser Ile Ser Asp			
145	150	155	160
aat ggc tat cag ttc atg ctg act gat cgg gag aat cag tct atc ttt			528
Asn Ala Tyr Gln Phe Met Leu Thr Asp Arg Glu Asn Gln Ser Ile Leu			
165	170	175	
atc acc gga gaa tct ggc gca ggg aag act gtg aac acc aag cgt gtc			576
Ile Thr Gly Glu Ser Gly Ala Gly Lys Thr Val Asn Thr Lys Arg Val			
180	185	190	
atc cag tac ttt gca aca att gca gtt act ggg gag aag aag aag gaa			624
Ile Gln Tyr Phe Ala Thr Ile Ala Val Thr Gly Glu Lys Lys Lys Glu			
195	200	205	
gaa gtt act tct ggc aaa atg cag ggg act ctg gaa gat caa atc atc			672
Glu Val Thr Ser Gly Lys Met Gln Gly Thr Leu Glu Asp Gln Ile Ile			
210	215	220	
agt ggc aac ccc cta ctg gag gcc ttt ggc aac gcc aag acc gtg agg			720
Ser Ala Asn Pro Leu Leu Glu Ala Phe Gly Asn Ala Lys Thr Val Arg			
225	230	235	240
aat gac aac tcc tct cgc ttt ggt aaa ttc atc agg atc cac ttc ggt			768
Asn Asp Asn Ser Ser Arg Phe Gly Lys Phe Ile Arg Ile His Phe Gly			
245	250	255	
acc aca ggg aaa ctg gct tct gct gat att gaa aca tat ctt ctg gag			816
Thr Thr Gly Lys Leu Ala Ser Ala Asp Ile Glu Thr Tyr Leu Leu Glu			

260	265	270	
aag tat aga gtt act ttc cag cta aag gct gaa aga ago tat cat att			864
Lys Ser Arg Val Thr Phe Gln Leu Lys Ala Glu Arg Ser Tyr His Ile			
275	280	285	
ttt tat cag atc atg tct aac aag aag cca gat cta att gaa atg ctc			912
Phe Tyr Gln Ile Met Ser Asn Lys Lys Pro Asp Leu Ile Glu Met Leu			
290	295	300	
ctg atc aac aac aac cca tac gat tat gcc ttc gtc agt caa gag gag			960
Leu Ile Thr Thr Asn Pro Tyr Asp Tyr Ala Phe Val Ser Gln Gly Glu			
305	310	315	320
atc aca gtc ccc agc att gat gac caa gaa gag ttg atg gct aca gat			1008
Ile Thr Val Pro Ser Ile Asp Asp Gln Glu Glu Leu Met Ala Thr Asp			
325	330	335	
agt gcc att gaa att ctg gcc ttt act tca gat gaa aga gtg tcc atc			1056
Ser Ala Ile Glu Ile Leu Gly Phe Thr Ser Asp Glu Arg Val Ser Ile			
340	345	350	
tat aag ctc aca gag gct gtg atg cat tat gag aac atg aac ttc aag			1104
Tyr Lys Leu Thr Gly Ala Val Met His Tyr Gly Asn Met Lys Phe Lys			
355	360	365	
caa aag cag cgt gag gag caa gct gag cca gat gcc act gaa gtt gct			1152
Gln Lys Gln Arg Glu Glu Gln Ala Glu Pro Asp Gly Thr Glu Val Ala			
370	375	380	
gac aag gca gcc tat ctc caa aat ctg aac tct gca gat ctg ctc aac			1200
Asp Lys Ala Ala Tyr Leu Gln Asn Leu Asn Ser Ala Asp Leu Leu Lys			
385	390	395	400
gcc ctc tgc tac cct agg gtc aag gtc gcc aat gag tat gtc aac aac			1248
Ala Leu Cys Tyr Pro Arg Val Lys Val Gly Asn Glu Tyr Val Thr Lys			

405	410	415	
ggt caa act gtg cag cag gtg tac aat gca atg ggt gct ctg gcc aaa			1295
Gly Gln Thr Val Gln Gln Val Tyr Asn Ala Val Gly Ala Leu Ala Lys			
420	425	430	
gct gtc tac gat aag atg ttc ttg tgg atg gtc acc cgc atc aac cag			1344
Ala Val Tyr Asp Lys Met Phe Leu Trp Met Val Thr Arg Ile Asn Gln			
435	440	445	
cag ctg gac acc aag cag ccc agg cag tac ttc att gag gtc ttg gac			1392
Gln Leu Asp Thr Lys Gln Pro Arg Gln Tyr Phe Ile Gly Val Leu Asp			
450	455	460	
att ggt ggc ttt gag atc ttt gat ttc aac agc ctg gag cag ctg tgc			1440
Ile Ala Gly Phe Glu Ile Phe Asp Phe Asn Ser Leu Glu Gln Leu Cys			
465	470	475	480
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Ile Asn Phe Thr Asn Glu Lys Leu Gln Gln Phe Phe Asn His His Met			
485	490	495	
ttc gtc ctg gag cag gag gag tac aag aag gaa ggc att gag tgg acg			1536
Phe Val Leu Glu Glu Gln Glu Tyr Lys Lys Glu Gly Ile Glu Trp Thr			
500	505	510	
ttc att gac ttt gag atg gac ctg gct gcc tgc atc gag ctg atc gag			1584
Phe Ile Asp Phe Gly Met Asp Leu Ala Ala Cys Ile Glu Leu Ile Glu			
515	520	525	
aag cct atg ggc atc ttc tcc atc ctg gaa gag gag tgc atg ttc ccc			1632
Lys Pro Met Gly Ile Phe Ser Ile Leu Glu Glu Glu Cys Met Phe Pro			
530	535	540	
aag gag aca gac acc tcc ttc aag aac aag ctg tat gaa caa cat ett			1680
Lys Ala Thr Asp Thr Ser Phe Lys Asn Lys Leu Tyr Glu Gln His Leu			

545	550	555	560
gga aaa tcc aat aac ttc cag aag ccc aag cct gcc aaa ggc aag cct	1728		
Gly Lys Ser Asn Asn Phe Gln Lys Pro Lys Pro Ala Lys Gly Lys Pro			
565	570	575	
sag gcc cac ttc tct ttg att cac tat gct gcc acc gtg gac tac aac	1776		
Glu Ala His Phe Ser Leu Ile His Tyr Ala Gly Thr Val Asp Tyr Asn			
580	585	590	
att gcc ggc tgg ctt gac aag aac aag gac ccc ctg aat gag act gtg	1824		
Ile Ala Gly Trp Leu Asp Lys Asn Lys Asp Pro Leu Asn Glu Thr Val			
595	600	605	
gtg ggg ctg tac cag aag tct gca atg aag aat ctg gct ctc ctc ttt	1872		
Val Gly Leu Tyr Gln Lys Ser Ala Met Lys Thr Leu Ala Leu Leu Phe			
610	615	620	
gtt ggg gca aag gga ggc gaa gca gag gct gcc ggt gga aag aaa ggt	1920		
Val Gly Ala Thr Gly Ala Glu Ala Glu Ala Gly Gly Gly Lys Lys Gly			
625	630	635	640
ggt aag aag aag ggt tct tct ttc cag aat gtg tgg gct ctc ttc aag	1968		
Gly Lys Lys Lys Gly Ser Ser Phe Gln Thr Val Ser Ala Leu Phe Arg			
645	650	655	
gag aat ttg aat aag ctg atg acc aac ttg agg agc act cac ccc csc	2016		
Glu Asn Leu Asn Lys Leu Met Thr Asn Leu Arg Ser Thr His Pro His			
660	665	670	
ttt gtg cgg tgc atc atc ccc aat gaa act aaa aat cct ggt gcc atg	2064		
Phe Val Arg Cys Ile Ile Pro Asn Glu Thr Lys Thr Pro Gly Ala Met			
675	680	685	
gag aat gag att gtc ctg aat cag ctg agg tgt aac ggt gtg ctg gaa	2112		
Glu His Glu Leu Val Leu His Gln Leu Arg Cys Asn Gly Val Leu Glu			

690	695	700	
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Gly Ile Arg Ile Cys Arg Lys Gly Phe Pro Ser Arg Ile Leu Tyr Ala			
705	710	715	720
gac ttc aaa cag aga tac aag gtg tta aat gca agt gct atc cct gaa	2208		
Asp Phe Lys Gln Arg Tyr Lys Val Leu Asn Ala Ser Ala Ile Pro Glu			
725	730	735	
gga caa ttc atc gat agc aag aag gct tca gag aag ctc ctg ggg tcc	2256		
Gly Gln Phe Ile Asp Ser Lys Lys Ala Ser Glu Lys Leu Leu Gly Ser			
740	745	750	
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755	760	765	
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Phe Lys Ala Gly Leu Leu Gly Leu Leu Glu Glu Met Arg Asp Glu Lys			
770	775	780	
ctg gcc cag ctg att acc cga acc cag gcc atg tgc aga ggg ttc ttg	2400		
Leu Ala Gln Leu Ile Thr Arg Thr Gln Ala Met Cys Arg Gly Phe Leu			
785	790	795	800
gca aga gtg gag tac cag aaa atg gtg gaa aga aga gag tcc atc ttc	2448		
Ala Arg Val Glu Tyr Gln Lys Met Val Glu Arg Arg Glu Ser Ile Phe			
805	810	815	
tac atc cag tac aat gtc cgt gcc ttc atg aat gtc aag cac tgg ccc	2496		
Cys Ile Gln Tyr Asn Val Arg Ala Phe Met Asn Val Lys His Trp Pro			
820	825	830	
tag atg aag ctg tat ttc aag atc aaa ccc ctc ctc aaa agt gca gag	2544		
Trp Met Lys Leu Tyr Phe Lys Ile Lys Pro Leu Leu Lys Ser Ala Glu			

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Thr Glu Lys Glu Met Ala Asn Met Lys Glu Glu Phe Glu Lys Thr Lys			
850	855	860	
gaa gag atg gct aag acc gag gca aaa agg aaa gag atg gaa gaa aaa	2640		
Glu Glu Leu Ala Lys Thr Glu Ala Lys Arg Lys Glu Leu Glu Glu Lys			
865	870	875	880
atg gtg act atg atg caa gaa aac aat gac ttg caa ctc cag gtt caa	2688		
Met Val Thr Leu Met Gln Glu Lys Asn Asp Leu Gln Leu Gln Val Gln			
885	890	895	
gct gaa gct gac agc ttg gct gat gca gag gaa agg tgt gac cag cta	2736		
Ala Glu Ala Asp Ser Leu Ala Asp Ala Glu Glu Arg Cys Asp Gln Leu			
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atc aaa acc aaa atc cag cta gaa gcc aaa atc aaa gag gtg act gag	2784		
Ile Lys Thr Lys Ile Gln Leu Glu Ala Lys Ile Lys Glu Val Thr Glu			
915	920	925	
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Arg Ala Glu Asp Glu Glu Glu Ile Asn Ala Glu Leu Thr Ala Lys Lys			
930	935	940	
agg aaa atg gag gat gaa tgt tca gaa ctc aag aaa gac att gat gac	2880		
Arg Lys Leu Glu Asp Glu Cys Ser Glu Leu Lys Lys Asp Ile Asp Asp			
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att gag atg aca atg gcc aag gtt gag aag gag aaa cat gcc aca gaa	2928		
Leu Glu Leu Thr Leu Ala Lys Val Glu Lys Glu Lys His Ala Thr Glu			
965	970	975	
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Asn Lys Val Lys Asn Leu Thr Glu Glu Met Ala Gly Leu Asp Glu Thr			

980	985	990	
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Ile Ala Lys Leu Thr Lys Glu Lys	Lys Ala Leu Gln Glu	Ala His Gln	
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Gln Thr Leu Asp Asp Leu Gln	Ala Glu Glu Asp Lys	Val Asn Thr	
1010	1015	1020	
ctg acc aaa gct aac atc aaa	ctt gaa caa caa gtg	gat gat ctt	3114
Leu Thr Lys Ala Lys Ile Lys	Leu Glu Gln Gln Val	Asp Asp Leu	
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gaa gaa tct ttg gaa caa gaa	aag aac atc cgg atg	gat cta gaa	3159
Glu Gly Ser Leu Glu Gln Glu	Lys Lys Ile Arg Met	Asp Leu Glu	
1040	1045	1050	
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Arg Ala Lys Arg Lys Leu Glu	Gly Asp Leu Lys Leu	Ala Gln Glu	
1055	1060	1065	
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Ser Ala Met Asp Ile Glu Asn	Asp Lys Gln Gln Leu	Asp Glu Lys	
1070	1075	1080	
ctt aac aag aac gag ttt gaa	atg agc ggt ctg caa	agc aag att	3294
Leu Lys Lys Lys Glu Phe Glu	Met Ser Gly Leu Gln	Ser Lys Ile	
1085	1090	1095	
gaa gat gaa caa gcc cit ggt	atg cag ctg cag aag	aaa atc aag	3339
Glu Asp Glu Gln Ala Leu Gly	Met Gln Leu Gln Lys	Lys Ile Lys	
1100	1105	1110	
gag tta caa gcc cgc att gag	gag ctg gag gag gaa	atc gag gca	3384
Glu Leu Gln Ala Arg Ile Glu	Glu Leu Glu Glu Glu	Ile Glu Ala	

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Glu Arg Ala Ser Arg Ala Lys	Ala Glu Lys Gln Arg	Ser Asp Leu	
1130	1135	1140	
tcc cgg gag ctg gag gag atc	agt gag aag ctg gaa	gaa gcc ggt	3474
Ser Arg Glu Leu Glu Glu Ile	Ser Glu Arg Leu Glu	Glu Ala Gly	
1145	1150	1155	
gag gcc acc tcc gcc cag att	gag atg aac aag aag	cgg gaa gct	3519
Gly Ala Thr Ser Ala Gln Ile	Glu Met Asn Lys Lys	Arg Glu Ala	
1160	1165	1170	
gag ttc cag aac atg cgc agc	gac ctg gag gag gcc	acc cta cag	3564
Glu Phe Gln Lys Met Arg Arg	Asp Leu Glu Glu Ala	Thr Leu Gln	
1175	1180	1185	
cat gag gcc aac gcc gcc acc	ctg agc aag aag cat	gca gat agt	3609
His Glu Ala Thr Ala Ala Thr	Leu Arg Lys Lys His	Ala Asp Ser	
1190	1195	1200	
gtg gcc gag ctt gag gag cag	att gac aac ctg cag	cga gtg aag	3654
Val Ala Glu Leu Gly Glu Gln	Ile Asp Asn Leu Gln	Arg Val Lys	
1205	1210	1215	
cag aag ctg gag aag gag aag	agt gag atg aag atg	gag atc gat	3699
Gln Lys Leu Glu Lys Glu Lys	Ser Glu Met Lys Met	Glu Ile Asp	
1220	1225	1230	
gac ctt gct agt aac atg gag	act gtc tcc aac gcc	aag gga aac	3744
Asp Leu Ala Ser Asn Met Glu	Thr Val Ser Lys Ala	Lys Gly Asn	
1235	1240	1245	
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Leu Glu Lys Met Cys Arg Ala	Leu Glu Asp Gln Leu	Ser Glu Ile	

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Lys Thr	Lys Glu Glu Glu Gln	Gln Arg Leu Ile Asn	Asp Leu Thr
1265	1270	1275	
gca cag	aga gag cgc ctg caa	aca gaa tca ggt gaa	tat tca cgc 3879
Ala Gln	Arg Ala Arg Leu Gln	Thr Glu Ser Gly Glu	Tyr Ser Arg
1280	1285	1290	
cag cta	gat gaa aag gac aca	cta gtt tca cag ctc	tgg agg ggc 3924
Gln Leu	Asp Glu Lys Asp Thr	Leu Val Ser Gln Leu	Ser Arg Gly
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aaa caa	gac ttt act caa cag	att gag gaa ctg aaa	agg caa ctt 3969
Lys Gln	Ala Phe Thr Gln Gln	Ile Glu Glu Leu Lys	Arg Gln Leu
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gaa gag	gag ata aag gcc aag	agt gcc ctg gca cat	gcc ctg cag 4014
Glu Glu	Glu Ile Lys Ala Lys	Ser Ala Leu Ala His	Ala Leu Gln
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tcc tcc	cgc cat gac tgt gac	ctg ctg cgg gaa cag	tat gag gag 4059
Ser Ser	Arg His Asp Cys Asp	Leu Leu Arg Glu Gln	Tyr Glu Glu
1340	1345	1350	
gag cag	gaa gcc aag gcc gag	cta cag aga gca atg	tcc aag gcc 4104
Glu Gln	Glu Ala Lys Ala Glu	Leu Gln Arg Ala Met	Ser Lys Ala
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aac agt	gag gtt gcc cag tgg	agg acc aaa tat gag	aca gat gcc 4149
Asn Ser	Glu Val Ala Gln Trp	Arg Thr Lys Tyr Glu	Thr Asp Ala
1370	1375	1380	
atc cag	cgc aca gag gag ctg	gag gag gcc aag aag	aag ctg gct 4194
Ile Gln	Arg Thr Glu Glu Leu	Glu Glu Ala Lys Lys	Lys Leu Ala

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cag cgt ctg cag gat gct gag gaa cat gta gaa gct gtg aat gaa			4239
Gln Arg Leu Gln Asp Ala Glu Glu His Val Glu Ala Val Asn Ala			
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aaa tgt gct tcc ctt gag aag acg sag cag agg ctc cag aat gaa			4284
Lys Cys Ala Ser Leu Glu Lys Thr Lys Gln Arg Leu Gln Asn Glu			
1415	1420	1425	
gtt gag gac ctc atg att gat gtt sag aag aca aat gct gcc tgt			4329
Val Glu Asp Leu Met Ile Asp Val Glu Arg Thr Asn Ala Ala Cys			
1430	1435	1440	
gcc gcc ctg gac aaa aag caa agg aac ttt gat aag atc ctg gca			4374
Ala Ala Leu Asp Lys Lys Gln Arg Asn Phe Asp Lys Ile Leu Ala			
1445	1450	1455	
gaa tag aac cag aag tgt gaa gaa aat cat gct gaa ctt gaa gct			4419
Glu Trp Lys Gln Lys Cys Glu Glu Thr His Ala Glu Leu Glu Ala			
1460	1465	1470	
tct caa aag gaa tcc cgc tca ctc agc aca gaa cta ttt aag att			4464
Ser Gln Lys Glu Ser Arg Ser Leu Ser Thr Glu Leu Phe Lys Ile			
1475	1480	1485	
aag aat gct tat gag gaa tct tta gac caa ctt gaa acc ttg aac			4509
Lys Asn Ala Tyr Glu Glu Ser Leu Asp Gln Leu Glu Thr Leu Lys			
1490	1495	1500	
cag gaa aat sag aat ctg cag cag gag att tct gat ctc aat gaa			4554
Arg Glu Asn Lys Asn Leu Gln Gln Glu Ile Ser Asp Leu Thr Glu			
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cag att gaa gaa gaa gaa aag cgc atc cat gaa ctg gaa aac ata			4599
Gln Ile Ala Glu Gly Gly Lys Arg Ile His Glu Leu Glu Lys Ile			

1520	1525	1530	
aag aag caa gtt gag caa gaa aag tet gaa ctt cag got ggc ita			4644
Lys Lys Gln Val Glu Gln Glu Lys Ser Glu Leu Gln Ala Ala Leu			
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gag gag gca gag gca tet ctt gaa cat gaa gag aga aag atc ctg			4689
Glu Glu Ala Glu Ala Ser Leu Glu His Glu Glu Gly Lys Ile Leu			
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ggc atc cag ctt gag ttg aac caa gtc aag tet gag att gat aag			4734
Arg Ile Gln Leu Glu Leu Asn Gln Val Lys Ser Glu Val Asp Arg			
1565	1570	1575	
aaa att gct gaa aaa gat gag gaa att gac cag atg aag aga aac			4779
Lys Ile Ala Glu Lys Asp Glu Glu Ile Asp Gln Met Lys Arg Asn			
1580	1585	1590	
cac att aga atc gtg gag tcc atg cag agc aca ctg gat got gag			4824
His Ile Arg Ile Val Glu Ser Met Gln Ser Thr Leu Asp Ala Glu			
1595	1600	1605	
atc agc agc agc aat gat gcc att agg ctc aag aag aag atg gag			4869
Ile Arg Ser Arg Asn Asp Ala Ile Arg Leu Lys Lys Lys Met Glu			
1610	1615	1620	
gga gac ctc aat gaa atg gaa atc cag ctg aac cat gcc aac cgc			4914
Gly Asp Leu Asn Glu Met Glu Ile Gln Leu Asn His Ala Asn Arg			
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atg got got gag gcc ctg agg aac tat agg aac acc caa gcc atc			4959
Met Ala Ala Glu Ala Leu Arg Asn Tyr Arg Asn Thr Gln Ala Ile			
1640	1645	1650	
ctc aag gat acc cag ctc cac cta gat gat got ctc egg agc caa			5004
Leu Lys Asp Thr Gln Leu His Leu Asp Asp Ala Leu Arg Ser Gln			

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Glu Asp Leu Lys Glu Gln Leu Ala Met Val Glu Arg Arg Ala Asn			
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ctg ctg cag gct gag atc gag gaa cta cga gcc aat ctg gaa cag			5094
Leu Leu Gln Ala Glu Ile Glu Glu Leu Arg Ala Thr Leu Glu Gln			
1685	1690	1695	
acc gag agc agc aag aaa atc gca gaa cag gag ctc ctg gat gcc			5139
Thr Glu Arg Ser Arg Lys Ile Ala Glu Gln Glu Leu Leu Asp Ala			
1700	1705	1710	
agt gaa cgt gtt cag ctc ctg gac acc cag aac acc agc ctg atc			5184
Ser Glu Arg Val Gln Leu Leu His Thr Gln Asn Thr Ser Leu Ile			
1715	1720	1725	
aac acc aag aag aag ctg gag gca gac att tcc caa atc cag gga			5229
Asn Thr Lys Lys Lys Leu Glu Thr Asp Ile Ser Gln Ile Gln Gly			
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gag atg gaa gac atc atc cag gaa gcc cgc aat gca gaa gag aag			5274
Glu Met Glu Asp Ile Ile Gln Glu Ala Arg Asn Ala Glu Glu Lys			
1745	1750	1755	
gcc aag aag gcc atc aat gat gct gcc atg atg gct gag gag ctg			5319
Ala Lys Lys Ala Ile Thr Asp Ala Ala Met Met Ala Glu Glu Leu			
1760	1765	1770	
aag aag gaa cag gac acc agc gcc cat ctg gag cgg atg aag aag			5364
Lys Lys Glu Gln Asp Thr Ser Ala His Leu Glu Arg Met Lys Lys			
1775	1780	1785	
aac tlg gaa cag aag gta aag gac ctg cag cat cgt ctg gat gag			5409
Asn Leu Glu Gln Thr Val Lys Asp Leu Gln His Arg Leu Asp Glu			

1790	1795	1800	
gct gag cag ctg gcc ctg aag ggt gag aag aag cag atc cag aaa			5454
Ala Glu Gln Leu Ala Leu Lys Gly Gly Lys Lys Gln Ile Gln Lys			
1805	1810	1815	
ctg gag gcc agg gtt cgt gaa ctt gaa ggt gaa gtt gaa agt gaa			5499
Leu Glu Ala Arg Val Arg Glu Leu Glu Gly Glu Val Glu Ser Glu			
1820	1825	1830	
cag aag cgc aat gtt gaa gct gtc aag ggt cta cgc aaa cat gag			5544
Gln Lys Arg Asn Val Glu Ala Val Lys Gly Leu Arg Lys His Glu			
1835	1840	1845	
aga aaa gtg aag gaa ctc act tac caa act gag gaa gac cgc aag			5589
Arg Lys Val Lys Glu Leu Thr Tyr Gln Thr Glu Glu Asp Arg Lys			
1850	1855	1860	
aat att ctc agg ctg cag gac ctg gtg gac aag ctg caa gca aag			5634
Asn Ile Leu Arg Leu Gln Asp Leu Val Asp Lys Leu Gln Ala Lys			
1865	1870	1875	
gtg aaa tcc tac aag aga caa gct gaa gaa gag gag gaa caa tcc			5679
Val Lys Ser Tyr Lys Arg Gln Ala Glu Glu Ala Glu Glu Gln Ser			
1880	1885	1890	
aac gtc aac ctc tcc aaa ttc cgg agg atc cag cac gag ctg gag			5724
Asn Val Asn Leu Ser Lys Phe Arg Arg Ile Gln His Glu Leu Glu			
1895	1900	1905	
gag gcc gag gaa agg gct gac att gct gag tcc cag gtc aac aag			5769
Glu Ala Glu Glu Arg Ala Asp Ile Ala Glu Ser Gln Val Asn Lys			
1910	1915	1920	
ctg agg gtg aag agc agg gag gtt cac aca aaa atc ata agt gaa			5814
Leu Arg Val Lys Ser Arg Glu Val His Thr Lys Ile Ile Ser Glu			

1925

1930

1935

gag taa ttatctatcc tgcctgaagg tgcctcaaga aaagcacaag atgtgaaat 5870
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35 40 45

Lys Ala Thr Val Gln Ser Arg Glu Gly Gly Lys Val Thr Ala Lys Thr
50 55 60

Glu Ala Gly Ala Thr Val Thr Val Lys Asp Asp Gln Val Phe Pro Met
65 70 75 80

Asn Pro Pro Lys Tyr Asp Lys Ile Glu Asp Met Ala Met Met Thr His
85 90 95

Leu His Glu Pro Ala Val Leu Tyr Asn Leu Lys Glu Arg Tyr Ala Ala
100 105 110

Trp Met Ile Tyr Thr Tyr Ser Gly Leu Phe Cys Val Thr Val Asn Pro
115 120 125

Tyr Lys Trp Leu Pro Val Tyr Asn Ala Glu Val Val Thr Ala Tyr Arg
130 135 140

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145 150 155 160

Asn Ala Tyr Gln Phe Met Leu Thr Asp Arg Glu Asn Gln Ser Ile Leu
165 170 175

Ile Thr Gly Glu Ser Gly Ala Gly Lys Thr Val Asn Thr Lys Arg Val
180 185 190

Ile Gln Tyr Phe Ala Thr Ile Ala Val Thr Gly Glu Lys Lys Lys Glu
195 200 205

Glu Val Thr Ser Gly Lys Met Gln Gly Thr Leu Glu Asp Gln Ile Ile
210 215 220

Ser Ala Asn Pro Leu Leu Glu Ala Phe Gly Asn Ala Lys Thr Val Arg
225 230 235 240

Asn Asp Asn Ser Ser Arg Phe Gly Lys Phe Ile Arg Ile His Phe Gly
245 250 255

Thr Thr Gly Lys Leu Ala Ser Ala Asp Ile Glu Thr Tyr Leu Leu Glu
260 265 270

Lys Ser Arg Val Thr Phe Gln Leu Lys Ala Glu Arg Ser Tyr His Ile
275 280 285

Phe Tyr Gln Ile Met Ser Asn Lys Lys Pro Asp Leu Ile Glu Met Leu
290 295 300

Leu Ile Thr Thr Asn Pro Tyr Asp Tyr Ala Phe Val Ser Gln Gly Glu
305 310 315 320

Ile Thr Val Pro Ser Ile Asp Asp Gln Glu Glu Leu Met Ala Thr Asp
325 330 335

Ser Ala Ile Glu Ile Leu Gly Phe Thr Ser Asp Glu Arg Val Ser Ile
340 345 350

Tyr Lys Leu Thr Gly Ala Val Met His Tyr Gly Asn Met Lys Phe Lys
355 360 365

Gln Lys Gln Arg Glu Glu Gln Ala Glu Pro Asp Gly Thr Glu Val Ala
370 375 380

Asp Lys Ala Ala Tyr Leu Gln Asn Leu Asn Ser Ala Asp Leu Leu Lys
385 390 395 400

Ala Leu Cys Tyr Pro Arg Val Lys Val Gly Asn Glu Tyr Val Thr Lys
405 410 415

Gly Gln Thr Val Gln Gln Val Tyr Asn Ala Val Gly Ala Leu Ala Lys
420 425 430

Ala Val Tyr Asp Lys Met Phe Leu Trp Met Val Thr Arg Ile Asn Gln
435 440 445

Gln Leu Asp Thr Lys Gln Pro Arg Gln Tyr Phe Ile Gly Val Leu Asp
450 455 460

Ile Ala Gly Phe Glu Ile Phe Asp Phe Asn Ser Leu Glu Gln Leu Cys
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Ile Asn Phe Thr Asn Glu Lys Leu Gln Gln Phe Phe Asn His His Met
485 490 495

Phe Val Leu Glu Gln Glu Glu Tyr Lys Lys Glu Gly Ile Glu Trp Thr
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Phe Ile Asp Phe Gly Met Asp Leu Ala Ala Cys Ile Glu Leu Ile Glu
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Lys Pro Met Gly Ile Phe Ser Ile Leu Glu Glu Glu Cys Met Phe Pro
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Lys Ala Thr Asp Thr Ser Phe Lys Asn Lys Leu Tyr Glu Gln His Leu
545 550 555 560

Gly Lys Ser Asn Asn Phe Gln Lys Pro Lys Pro Ala Lys Gly Lys Pro
565 570 575

Glu Ala His Phe Ser Leu Ile His Tyr Ala Gly Thr Val Asp Tyr Asn
580 585 590

Ile Ala Gly Trp Leu Asp Lys Asn Lys Asp Pro Leu Asn Glu Thr Val
595 600 605

Val Gly Leu Tyr Gln Lys Ser Ala Met Lys Thr Leu Ala Leu Leu Phe
610 615 620

Val Gly Ala Thr Gly Ala Glu Ala Glu Ala Gly Gly Gly Lys Lys Gly
625 630 635 640

Gly Lys Lys Lys Gly Ser Ser Phe Gln Thr Val Ser Ala Leu Phe Arg
645 650 655

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Phe Val Arg Cys Ile Ile Pro Asn Glu Thr Lys Thr Pro Gly Ala Met
675 680 685

Glu His Glu Leu Val Leu His Gln Leu Arg Cys Asn Gly Val Leu Glu
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Gly Ile Arg Ile Cys Arg Lys Gly Phe Pro Ser Arg Ile Leu Tyr Ala
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Asp Phe Lys Gln Arg Tyr Lys Val Leu Asn Ala Ser Ala Ile Pro Glu
725 730 735

Gly Gln Phe Ile Asp Ser Lys Lys Ala Ser Glu Lys Leu Leu Gly Ser
740 745 750

Ile Asp Ile Asp His Thr Gln Tyr Lys Phe Gly His Thr Lys Val Phe
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Cys Ile Gln Tyr Asn Val Arg Ala Phe Met Asn Val Lys His Trp Pro
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900 905 910

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915 920 925

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930 935 940

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Ile Ala Lys Leu Thr Lys Glu Lys Lys Ala Leu Gln Glu Ala His Gln
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Glu Phe Gln Lys Met Arg Arg Asp Leu Glu Glu Ala Thr Leu Gln
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Ile Glu Ala Thr Ala Ala Thr Leu Arg Lys Lys His Ala Asp Ser
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1565 1570 1575

Lys Ile Ala Glu Lys Asp Glu Glu Ile Asp Gln Met Lys Arg Asn
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His Ile Arg Ile Val Glu Ser Met Gln Ser Thr Leu Asp Ala Glu
1595 1600 1605

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Ala Lys Lys Ala Ile Thr Asp Ala Ala Met Met Ala Glu Glu Leu
1760 1765 1770

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1775 1780 1785

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1805 1810 1815

Leu Glu Ala Arg Val Arg Glu Leu Glu Gly Glu Val Glu Ser Glu
1820 1825 1830

Gln Lys Arg Asn Val Glu Ala Val Lys Gly Leu Arg Lys His Glu
1835 1840 1845

Arg Lys Val Lys Glu Leu Thr Tyr Gln Thr Glu Glu Asp Arg Lys
1850 1855 1860

Asn Ile Leu Arg Leu Gln Asp Leu Val Asp Lys Leu Gln Ala Lys
1865 1870 1875

Val Lys Ser Tyr Lys Arg Gln Ala Glu Glu Ala Glu Glu Gln Ser
1880 1885 1890

Asn Val Asn Leu Ser Lys Phe Arg Arg Ile Gln His Glu Leu Glu
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 Trp Lys His Lys Gly Arg Asp Val Ile Leu Lys Lys Asp Val Arg Phe
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aca tat gta gag aac cag act gcc atg gaa tta gag gag cag gtc act 1015

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atg gtg gtg cgt agc cat gcc cgt gtg tcc tcc ctg acc ctg aag agc 1159
 Met Val Val Arg Ser His Ala Arg Val Ser Ser Leu Thr Leu Lys Ser
 360 365 370

atc cag tac act gat gcc gga gag tac atc tgc acc gcc agc aac acc 1207
 Ile Gln Tyr Thr Asp Ala Gly Glu Tyr Ile Cys Thr Ala Ser Asn Thr
 375 380 385 390

atc gcc cag gac tcc cag tcc atg tac ctt gaa gtg caa tat gcc cca 1255
 Ile Gly Gln Asp Ser Gln Ser Met Tyr Leu Glu Val Gln Tyr Ala Pro
 395 400 405

aag cta cag gcc cct gtg gct gtg tac act tgg gag ggg aac cag gtg 1303
 Lys Leu Gln Gly Pro Val Ala Val Tyr Thr Trp Glu Gly Asn Gln Val
 410 415 420

aac atc acc tgc gag gta ttt gcc tat ccc agt gcc acc atc tcc tgg 1351
 Asn Ile Thr Cys Glu Val Phe Ala Tyr Pro Ser Ala Thr Ile Ser Trp
 425 430 435

ttt cag gat gcc cag ctg ctg ccc agc tcc aat tac agc aat atc aag 1399
 Phe Arg Asp Gly Gln Leu Leu Pro Ser Ser Asn Tyr Ser Asn Ile Lys
 440 445 450

atc tac aac acc ccc tct gcc agc tat ctg gag gtg acc cca gac tct 1447

Ile Tyr Asn Thr Pro Ser Ala Ser Tyr Leu Glu Val Thr Pro Asp Ser
 455 460 465 470
 gag aat gat ttt ggg aac tac aac tgt aat gaa gtg aac cgc att ggg 1495
 Glu Asn Asp Phe Gly Asn Tyr Asn Cys Thr Ala Val Asn Arg Ile Gly
 475 480 485
 cag gag tcc ttc gaa ttc atc ctt gtt aca gaa gac aac ccc tct tca 1543
 Gln Glu Ser Phe Glu Phe Ile Leu Val Gln Ala Asp Thr Pro Ser Ser
 490 495 500
 cca tcc atc gac cag gtg gag cca tac tcc agc aca gcc cag gtg cag 1591
 Pro Ser Ile Asp Gln Val Glu Pro Tyr Ser Ser Thr Ala Gln Val Gln
 505 510 515
 ttt gat gaa cca gag gcc aca ggt ggg gtg ccc atc ctc aca tac aca 1639
 Phe Asp Glu Pro Glu Ala Thr Gly Gly Val Pro Ile Leu Lys Tyr Lys
 520 525 530
 gct gag tag aga gaa gtg ggt gaa gaa gta tgg cat tcc aag tgg tat 1687
 Ala Glu Trp Arg Ala Val Gly Glu Glu Val Trp His Ser Lys Trp Tyr
 535 540 545 550
 gat gcc aag gaa gcc agc atg gag ggc atc gtc acc atc gtg gcc ctg 1735
 Asp Ala Lys Glu Ala Ser Met Glu Gly Ile Val Thr Ile Val Gly Leu
 555 560 565
 aag ccc gaa aca acg tac gcc gta agg ctg gag gag ctc aat gcc aca 1783
 Lys Pro Glu Thr Thr Tyr Ala Val Arg Leu Ala Ala Leu Asn Gly Lys
 570 575 580
 ggg ctg ggt gag atc agc gag gcc tcc gag ttc aag acg cag cca gtc 1831
 Gly Leu Gly Glu Ile Ser Ala Ala Ser Glu Phe Lys Thr Gln Pro Val
 585 590 595
 caa ggg gaa ccc agt gca cct aag ctc gaa ggg cag atg gga gag gat 1879

Gln Gly Glu Pro Ser Ala Pro Lys Leu Glu Gly Gln Met Gly Glu Asp
 600 605 610
 gga aac tat att aas gtg aac ctg atc aag cag gat gac ggc ggc tcc 1927
 Gly Asn Ser Ile Lys Val Asn Leu Ile Lys Gln Asp Asp Gly Gly Ser
 615 620 625 630
 ccc atc aga cac tat ctg gtc agg tac cga gag ctc tcc tcc gag tgg 1975
 Pro Ile Arg His Tyr Leu Val Arg Tyr Arg Ala Leu Ser Ser Glu Trp
 635 640 645
 aas cca gag atc agg ctc cag tat ggc agt gac cac gtc atg ctg aag 2023
 Lys Pro Glu Ile Arg Leu Pro Ser Gly Ser Asp His Val Met Leu Lys
 650 655 660
 tcc ctg gac tgg aat gct gag tat gag gtc tac gtg gtg gct gag aac 2071
 Ser Leu Asp Trp Asn Ala Glu Tyr Glu Val Tyr Val Val Ala Glu Asn
 665 670 675
 cag caa gaa aas tcc aag gag gct cat ttt gtg ttc agg acc tcc gcc 2119
 Gln Gln Gly Lys Ser Lys Ala Ala His Phe Val Phe Arg Thr Ser Ala
 680 685 690
 cag ccc aca gcc atc cca gcc aac ggc agc ccc acc tca ggc ctg agc 2167
 Gln Pro Thr Ala Ile Pro Ala Asn Gly Ser Pro Thr Ser Gly Leu Ser
 695 700 705 710
 acc ggg gcc atc gtg ggc atc ctc atc gtc atc ttc gtc ctg ctc ctg 2215
 Thr Gly Ala Ile Val Gly Ile Leu Ile Val Ile Phe Val Leu Leu Leu
 715 720 725
 gtg gtt gtg gac atc acc tgc tac ttc ctg aac aag tgt ggc ctg ttc 2263
 Val Val Val Asp Ile Thr Cys Tyr Phe Leu Asn Lys Cys Gly Leu Phe
 730 735 740
 atg tgc att gag gtc aac ctg tgt gga aas gcc ggg ccc ggg gcc aag 2311

Met Cys Ile Ala Val Asn Leu Cys Gly Lys Ala Gly Pro Gly Ala Lys
745 750 755

ggc aag gac atg gag gag ggc aag gcc gcc ttc tgg aac gat gag tcc 2359
Gly Lys Asp Met Glu Glu Gly Lys Ala Ala Phe Ser Lys Asp Glu Ser
760 765 770

aag gag ccc atc gtg gag gtt cga acg gag gag gag agg acc cca aac 2407
Lys Glu Pro Ile Val Glu Val Arg Thr Glu Glu Glu Arg Thr Pro Asn
775 780 785 790

cat gat gga gag aaa cac acc gag ccc aac gag acc acg cca ctg aag 2455
His Asp Gly Gly Lys His Thr Glu Pro Asn Glu Thr Thr Pro Leu Thr
795 800 805

gag ccc gag aag ggc ccc gta gaa gcc aag cca gag tgc cag gag aca 2503
Glu Pro Glu Lys Gly Pro Val Glu Ala Lys Pro Glu Cys Gln Glu Thr
810 815 820

gaa acg aag cca gag cca gcc gaa gtc aag acg gtc ccc aat gac gcc 2551
Glu Thr Lys Pro Ala Pro Ala Glu Val Lys Thr Val Pro Asn Asp Ala
825 830 835

aca cag aca aag gag aac gag agc aac gca tga tgggtgaaga gaaccgagca 2604
Thr Gln Thr Lys Glu Asn Glu Ser Lys Ala
840 845

aagatcaaaa taataaagta cacagcaga 2633

<210> 8

<211> 848

<212> PRT

<213> Homo sapiens

<400> 8

Met Leu Glu Thr Lys Asp Leu Ile Trp Thr Leu Phe Phe Leu Gly Thr
1 5 10 15

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20 25 30

Val Gly Glu Ser Lys Phe Phe Leu Cys Glu Val Ala Gly Asp Ala Lys
35 40 45

Asp Lys Asp Ile Ser Trp Phe Ser Pro Asn Gly Glu Lys Leu Thr Pro
50 55 60

Asn Glu Glu Arg Ile Ser Val Val Trp Asn Asp Asp Ser Ser Ser Thr
65 70 75 80

Leu Thr Ile Tyr Asn Ala Asn Ile Asp Asp Ala Gly Ile Tyr Lys Cys
85 90 95

Val Val Thr Gly Glu Asp Gly Ser Glu Ser Glu Ala Thr Val Asn Val
100 105 110

Lys Ile Phe Glu Lys Leu Met Phe Lys Asn Ala Pro Thr Pro Glu Glu
115 120 125

Phe Arg Glu Gly Glu Asp Ala Val Ile Val Cys Asp Val Val Ser Ser
130 135 140

Leu Pro Pro Thr Ile Ile Trp Lys His Lys Gly Arg Asp Val Ile Leu
145 150 155 160

Lys Lys Asp Val Arg Phe Ile Val Leu Ser Asn Asn Tyr Leu Gln Ile
165 170 175

Arg Gly Ile Lys Lys Thr Asp Glu Gly Thr Tyr Arg Cys Glu Gly Arg
180 185 190

Ile Leu Ala Arg Gly Glu Ile Asn Phe Lys Asp Ile Gln Val Ile Val
195 200 205

Asn Val Pro Pro Thr Ile Arg Ala Arg Gln Asn Ile Val Asn Ala Thr
210 215 220

Ala Asn Leu Gly Gln Ser Val Thr Leu Val Cys Asp Ala Glu Arg Phe
225 230 235 240

Pro Glu Pro Thr Met Ser Trp Thr Lys Asp Gly Glu Gln Ile Glu Gln
245 250 255

Glu Glu Asp Asp Glu Lys Tyr Ile Phe Ser Asp Asp Ser Ser Gln Leu
260 265 270

Thr Ile Lys Lys Val Asp Lys Asn Asp Glu Ala Glu Tyr Ile Cys Ile
275 280 285

Ala Glu Asn Lys Ala Gly Glu Gln Asp Ala Thr Ile His Leu Lys Val
290 295 300

Phe Ala Lys Pro Lys Ile Thr Tyr Val Glu Asn Gln Thr Ala Met Glu
305 310 315 320

Leu Glu Glu Gln Val Thr Leu Thr Cys Glu Ala Ser Gly Asp Pro Ile
325 330 335

Pro Ser Ile Thr Trp Arg Thr Ser Thr Arg Asn Ile Ser Ser Glu Glu
340 345 350

Lys Thr Leu Asp Gly His Met Val Val Arg Ser His Ala Arg Val Ser
355 360 365

Ser Leu Thr Leu Lys Ser Ile Gln Tyr Thr Asp Ala Gly Glu Tyr Ile
370 375 380

Cys Thr Ala Ser Asn Thr Ile Gly Gln Asp Ser Gln Ser Met Tyr Leu
385 390 395 400

Glu Val Gln Tyr Ala Pro Lys Leu Gln Gly Pro Val Ala Val Tyr Thr
405 410 415

Trp Glu Gly Asn Gln Val Asn Ile Thr Cys Glu Val Phe Ala Tyr Pro
420 425 430

Ser Ala Thr Ile Ser Trp Phe Arg Asp Gly Gln Leu Leu Pro Ser Ser
435 440 445

Asn Tyr Ser Asn Ile Lys Ile Tyr Asn Thr Pro Ser Ala Ser Tyr Leu
450 455 460

Glu Val Thr Pro Asp Ser Glu Asn Asp Phe Gly Asn Tyr Asn Cys Thr
465 470 475 480

Ala Val Asn Arg Ile Gly Gln Glu Ser Phe Glu Phe Ile Leu Val Gln
485 490 495

Ala Asp Thr Pro Ser Ser Pro Ser Ile Asp Gln Val Glu Pro Tyr Ser
500 505 510

Ser Thr Ala Gln Val Gln Phe Asp Glu Pro Glu Ala Thr Gly Gly Val
515 520 525

Pro Ile Leu Lys Tyr Lys Ala Glu Trp Arg Ala Val Gly Glu Glu Val
530 535 540

Trp His Ser Lys Trp Tyr Asp Ala Lys Glu Ala Ser Met Glu Gly Ile
545 550 555 560

Val Thr Ile Val Gly Leu Lys Pro Glu Thr Thr Tyr Ala Val Arg Leu
565 570 575

Ala Ala Leu Asn Gly Lys Gly Leu Gly Glu Ile Ser Ala Ala Ser Glu
580 585 590

Phe Lys Thr Gln Pro Val Gln Gly Glu Pro Ser Ala Pro Lys Leu Glu
595 600 605

Gly Gln Met Gly Glu Asp Gly Asn Ser Ile Lys Val Asn Leu Ile Lys
610 615 620

Gln Asp Asp Gly Gly Ser Pro Ile Arg His Tyr Leu Val Arg Tyr Arg
625 630 635 640

Ala Leu Ser Ser Glu Trp Lys Pro Glu Ile Arg Leu Pro Ser Gly Ser
645 650 655

Asp His Val Met Leu Lys Ser Leu Asp Trp Asn Ala Glu Tyr Glu Val
660 665 670

Tyr Val Val Ala Glu Asn Gln Gln Gly Lys Ser Lys Ala Ala His Phe
675 680 685

Val Phe Arg Thr Ser Ala Gln Pro Thr Ala Ile Pro Ala Asn Gly Ser
690 695 700

Pro Thr Ser Gly Leu Ser Thr Gly Ala Ile Val Gly Ile Leu Ile Val
705 710 715 720

Ile Phe Val Leu Leu Leu Val Val Val Asp Ile Thr Cys Tyr Phe Leu
725 730 735

Asn Lys Cys Gly Leu Phe Met Cys Ile Ala Val Asn Leu Cys Gly Lys
740 745 750

Ala Gly Pro Gly Ala Lys Gly Lys Asp Met Glu Glu Gly Lys Ala Ala
755 760 765

Phe Ser Lys Asp Glu Ser Lys Glu Pro Ile Val Glu Val Arg Thr Glu
770 775 780

Glu Glu Arg Thr Pro Asn His Asp Gly Gly Lys His Thr Glu Pro Asn
785 790 795 800

Glu Thr Thr Pro Leu Thr Glu Pro Glu Lys Gly Pro Val Glu Ala Lys
805 810 815

Pro Glu Cys Gln Glu Thr Glu Thr Lys Pro Ala Pro Ala Glu Val Lys
820 825 830

Thr Val Pro Asn Asp Ala Thr Gln Thr Lys Glu Asn Glu Ser Lys Ala
835 840 845

<210> 9

<211> 1692

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (121)..(1080)

<400> 9

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gccactctct gcgcctggg ttggcgaaa gccaggacg tgcgcgcga ccgcaggat 120

atg gag cta ctg tgg cca ccg ctg cgc gac gta gac ctg acg gcc ccc 168
Met Glu Leu Leu Ser Pro Pro Leu Arg Asp Val Asp Leu Thr Ala Pro
1 5 10 15

gac ggc tat ctg tgc tcc ttt gcc acc acg gac gac ttc tat gac gac 216
Asp Gly Ser Leu Cys Ser Phe Ala Thr Thr Asp Asp Phe Tyr Asp Asp
20 25 30

ccg tgt ttc gac tcc ccg gac ctg cgc ttc ttc gaa gac ctg gac ccg 264
Pro Cys Phe Asp Ser Pro Asp Leu Arg Phe Phe Glu Asp Leu Asp Pro
35 40 45

cgc ctg atg cac gtg ggc ggc ctg ctg aca ccc gaa gag cac tgg cac 312
Arg Leu Met His Val Gly Ala Leu Leu Lys Pro Glu Glu His Ser His
50 55 60

ttc ccc ggc ggc gtg cac ccg gcc ccg ggc gca cat gag gac gag cat 360
Phe Pro Ala Ala Val His Pro Ala Pro Gly Ala Arg Glu Asp Glu His
65 70 75 80

gtg cgc gcg ccc agc ggg cac cac cag ggc ggc cgc tgc cta ctg tgg 408
Val Arg Ala Pro Ser Gly His His Gin Ala Gly Arg Cys Leu Leu Trp
85 90 95

gac tgc aag gac tgc aag cgc aag acc acc aac gcc gac cgc cgc aag	456
Ala Cys Lys Ala Cys Lys Arg Lys Thr Thr Asn Ala Asp Arg Arg Lys	
100 105 110	
gcc gcc acc atg cgc gag cgg cgc cgc atg acc aac gta aat gag gcc	504
Ala Ala Thr Met Arg Glu Arg Arg Arg Leu Ser Lys Val Asn Glu Ala	
115 120 125	
ttt gag aca ctc aag cgc tgc aag tgc acc aat cca aac cag cgc ttg	552
Phe Glu Thr Leu Lys Arg Cys Thr Ser Ser Asn Pro Asn Glu Arg Leu	
130 135 140	
ccc aag atg gag atc atg cgc aac gcc atc cgc tat atc gag gcc atg	600
Pro Lys Val Glu Ile Leu Arg Asn Ala Ile Arg Tyr Ile Glu Gly Leu	
145 150 155 160	
cag gct atg atg cgc gac cag gac gcc gcc ccc cct gcc gca gcc gcc	648
Gln Ala Leu Leu Arg Asp Gln Asp Ala Ala Pro Pro Gly Ala Ala Ala	
165 170 175	
tto tat gag cgc gcc cgc atg ccc cgc gcc cgc gcc gcc gag cac tac	696
Phe Tyr Ala Pro Gly Pro Leu Pro Pro Gly Arg Gly Gly Glu His Tyr	
180 185 190	
agg gcc gac tcc gac gcc tcc acc cgc cgc tcc aac tgc tcc gac gcc	744
Ser Gly Asp Ser Asp Ala Ser Ser Pro Arg Ser Asn Cys Ser Asp Gly	
195 200 205	
atg atg gac tac acc gcc ccc cgc acc gcc gcc cgg cgg cgg aac tgc	792
Met Met Asp Tyr Ser Gly Pro Pro Ser Gly Ala Arg Arg Arg Asn Cys	
210 215 220	
tac gaa gcc gcc tac tac aac gag gcc ccc acc gcc gaa ccc agg ccc gcc	840
Tyr Glu Gly Ala Tyr Tyr Asn Glu Ala Pro Ser Glu Pro Arg Pro Gly	
225 230 235 240	

aag agt gag gag gta tag agc cta gac tac ctg tcc agc atc gta gag 888
 Lys Ser Ala Ala Val Ser Ser Leu Asp Tyr Leu Ser Ser Ile Val Glu
 245 250 255

ggc atc tcc acc gag agc cct gag gag ccc gcc ctg ctg gag gag 936
 Arg Ile Ser Thr Glu Ser Pro Ala Ala Pro Ala Leu Leu Leu Ala Asp
 260 265 270

gta cct tct gag tag cct ccg cgc agc cca gag gct gcc gcc ccc agc 984
 Val Pro Ser Glu Ser Pro Pro Arg Arg Gln Glu Ala Ala Ala Pro Ser
 275 280 285

gag gaa gag agc agc gcc gac ccc acc cag tcc ccg gag gcc gcc ccc 1032
 Glu Gly Glu Ser Ser Gly Asp Pro Thr Gln Ser Pro Asp Ala Ala Pro
 290 295 300

cag tgc cct gag ggt gag acc ccc acc ccg ata tac cag gta ctg tga 1080
 Gln Cys Pro Ala Gly Ala Asn Pro Asn Pro Ile Tyr Gln Val Leu
 305 310 315

gagagatgta ggcgcacac ccgcacagg atggtgcct aggttcctc ggcgcacaaa 1140

gattgaactt aaatgcctcc ctcccaacag cgttttaaaa ggcctctctc ttgaggtagg 1200

agagcggag aactgaagtt tcgcgcctcc ccgcacaggc aaggacacag cgcggttttt 1260

tcacgcagc accctctctg gacacccatt gcgagggccg ctccgtgttc ctgggtggc 1320

cagagctaaa ccttagggg ataggttccg gttctcgcg ccttccatgg tgagccctc 1380

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accattttt gtaatactt ttgtactctc ttctgttaa taagagttc ttgcccagag 1560

aggagccctt ggggtgttat ttatctctaa gacaggtgtt gtgtgtctac agggatttg 1620

tacgtttata ccgacggcgg gcgagccggc ggcgtctgct caggtgatca aattaxagc 1680

gatsatttat aa 1692

<210> 10

<211> 319

<212> PRT

<213> Homo sapiens

<400> 10

Met	Glu	Leu	Leu	Ser	Pro	Pro	Leu	Arg	Asp	Val	Asp	Leu	Thr	Ala	Pro
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Asp	Gly	Ser	Leu	Cys	Ser	Phe	Ala	Thr	Thr	Asp	Asp	Phe	Tyr	Asp	Asp
			20					25						30	

Pro	Cys	Phe	Asp	Ser	Pro	Asp	Leu	Arg	Phe	Phe	Glu	Asp	Leu	Asp	Pro
		25					40					45			

Arg	Leu	Met	His	Val	Gly	Ala	Leu	Leu	Lys	Pro	Glu	Glu	His	Ser	His
	50					55					60				

Phe	Pro	Ala	Ala	Val	His	Pro	Ala	Pro	Gly	Ala	Arg	Glu	Asp	Glu	His
65					70					75				80	

Val	Arg	Ala	Pro	Ser	Gly	His	His	Gln	Ala	Gly	Arg	Cys	Leu	Leu	Trp
					85				90					95	

Ala Cys Lys Ala Cys Lys Arg Lys Thr Thr Asn Ala Asp Arg Arg Lys

100

105

110

Ala Ala Thr Met Arg Glu Arg Arg Arg Leu Ser Lys Val Asn Glu Ala

115

120

125

Phe Glu Thr Leu Lys Arg Cys Thr Ser Ser Asn Pro Asn Gln Arg Leu

130

135

140

Pro Lys Val Glu Ile Leu Arg Asn Ala Ile Arg Tyr Ile Glu Gly Leu

145

150

155

160

Gln Ala Leu Leu Arg Asp Gln Asp Ala Ala Pro Pro Gly Ala Ala Ala

165

170

175

Phe Tyr Ala Pro Gly Pro Leu Pro Pro Gly Arg Gly Gly Glu His Tyr

180

185

190

Ser Gly Asp Ser Asp Ala Ser Ser Pro Arg Ser Asn Cys Ser Asp Gly

195

200

205

Met Met Asp Tyr Ser Gly Pro Pro Ser Gly Ala Arg Arg Arg Asn Cys

210

215

220

Tyr Glu Gly Ala Tyr Tyr Asn Glu Ala Pro Ser Glu Pro Arg Pro Gly

225

230

235

240

Lys Ser Ala Ala Val Ser Ser Leu Asp Tyr Leu Ser Ser Ile Val Glu
 245 250 255

Arg Ile Ser Thr Glu Ser Pro Ala Ala Pro Ala Leu Leu Leu Ala Asp
 260 265 270

Val Pro Ser Glu Ser Pro Pro Arg Arg Gln Glu Ala Ala Ala Pro Ser
 275 280 285

Glu Gly Glu Ser Ser Gly Asp Pro Thr Gln Ser Pro Asp Ala Ala Pro
 290 295 300

Gln Cys Pro Ala Gly Ala Asn Pro Asn Pro Ile Tyr Gln Val Leu
 305 310 315

<210> 11

<211> 1427

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (43)..(810)

<400> 11

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 Met Asp Val Met

gat ggc tgc cag ttc tca cct tct gag tac ttc tac gac ggc tcc tgc 102
Asp Gly Cys Gln Phe Ser Pro Ser Glu Tyr Phe Tyr Asp Gly Ser Cys
5 10 15 20

ata cag tcc ccc gag ggt gaa ttt ggg gac gag ttt gtg cag cga atg 150
Ile Pro Ser Pro Glu Gly Glu Phe Gly Asp Glu Phe Val Pro Arg Val
25 30 35

gct gcc ttc gga ggc cac aaa gca gag ctg cag gcc tca gat gag gac 198
Ala Ala Phe Gly Ala His Lys Ala Glu Leu Gln Gly Ser Asp Glu Asp
40 45 50

gag cac gtg cga ggc cct acc gcc cac cac cag gct ggt cac tgc ctc 246
Glu His Val Arg Ala Pro Thr Gly His His Gln Ala Gly His Cys Leu
55 60 65

atg tgg gcc tgc aaa gcc tgc gag agg aag tcc acc acc atg gat cgg 294
Met Trp Ala Cys Lys Ala Cys Lys Arg Lys Ser Thr Thr Met Asp Arg
70 75 80

cgg aag gcc gcc act atg cgc gag cgg agg cgc ctg aag aag gtc aac 342
Arg Lys Ala Ala Thr Met Arg Glu Arg Arg Arg Leu Lys Lys Val Asn
85 90 95 100

cag gct ttc gaa acc ctc aag agg tgt acc aag acc aac ccc aac cag 390
Gln Ala Phe Glu Thr Leu Lys Arg Cys Thr Thr Thr Asn Pro Asn Gln
105 110 115

agg ctg ccc aag gtg gag atc ctc agg aat gcc atc cgc tac atc gag 438
Arg Leu Pro Lys Val Glu Ile Leu Arg Asn Ala Ile Arg Tyr Ile Glu
120 125 130

agg ctg cag gag ttg ctg aga gag cag gtg gag aac tac tat agc ctg 486
Ser Leu Gln Glu Leu Leu Arg Glu Gln Val Glu Asn Tyr Tyr Ser Leu
135 140 145

cag gga cag agc tgc tgc gag ccc acc agc ccc acc tcc aac tgc tct 534
 Pro Gly Gln Ser Cys Ser Glu Pro Thr Ser Pro Thr Ser Asn Cys Ser
 150 155 160

gat ggc atg ccc gaa tgt aac agt cct gtc tgg tcc aga aag agc agt 582
 Asp Gly Met Pro Glu Cys Asn Ser Pro Val Trp Ser Arg Lys Ser Ser
 165 170 175 180

act ttt gac agc atc tac tgt cct gat gta tca aat gta tat ggc acc 630
 Thr Phe Asp Ser Ile Tyr Cys Pro Asp Val Ser Asn Val Tyr Ala Thr
 185 190 195

gat aaa aac tcc tta tcc agc ttg gat tgc tta tcc aac aia gtg gac 678
 Asp Lys Asn Ser Leu Ser Ser Leu Asp Cys Leu Ser Asn Ile Val Asp
 200 205 210

cgg atc acc tcc tca gag caa cct ggg ttg cct ctc cag gat ctg got 726
 Arg Ile Thr Ser Ser Glu Gln Pro Gly Leu Pro Leu Gln Asp Leu Ala
 215 220 225

tat ctc tct cca gtt gcc agc acc gat tca cag cct cga act cca ggg 774
 Ser Leu Ser Pro Val Ala Ser Thr Asp Ser Gln Pro Arg Thr Pro Gly
 230 235 240

got tct agt tcc agg ctt atc tat cat gtc cta tga actaatcttc 820
 Ala Ser Ser Ser Arg Leu Ile Tyr His Val Leu
 245 250 255

tagctatata gacttcttcc agcagggcct aatacacagg accaagaagg cttaaaaaag 880

tcccaaacca agacaacatg tacataaaga tttcttttcc gtgttaaat tgtaaagatt 940

aacttgccac ttataaaga agtgtattta actaaaaagt catctttgca aataactctt 1000

tcttcttctt tcttcttctt tgcctagata ttaatacata gtccagtaa tactatttct 1060

gxtaggaggc cattgatgga aggtagcttg ttggaatgct taactatct atactatct 1120
 atatattata aatattgctc atcaaaatgt ctctgggtgt tagagcttts tttttttctt 1180
 taaacactta aacagcgtga gactcagita aatggaaatt taactatatt taactatttc 1240
 ttctctcttt aatcttttag ttatattgta ttaastssas atataactct gctaatgta 1300
 tatatttga tttttcttg taagaaatgt atctttttaa tgaagcaca aatagtact 1360
 ttgtggatca ttcaagata taagaaattt tggaaattcc accataaata aaatttttta 1420
 ctacaag 1427

<210> 12

<211> 255

<212> PRT

<213> Homo sapiens

<400> 12

Met Asp Val Met Asp Gly Cys Gln Phe Ser Pro Ser Glu Tyr Phe Tyr
 1 5 10 15

Asp Gly Ser Cys Ile Pro Ser Pro Glu Gly Glu Phe Gly Asp Glu Phe
 20 25 30

Val Pro Arg Val Ala Ala Phe Gly Ala His Lys Ala Glu Leu Gln Gly
 35 40 45

Ser Asp Glu Asp Glu His Val Arg Ala Pro Thr Gly His His Gln Ala

50

65

80

Gly His Cys Leu Met Trp Ala Cys Lys Ala Cys Lys Arg Lys Ser Thr

65

70

75

80

Thr Met Asp Arg Arg Lys Ala Ala Thr Met Arg Glu Arg Arg Arg Leu

85

90

95

Lys Lys Val Asn Gln Ala Phe Glu Thr Leu Lys Arg Cys Thr Thr Thr

100

105

110

Asn Pro Asn Gln Arg Leu Pro Lys Val Glu Ile Leu Arg Asn Ala Ile

115

120

125

Arg Tyr Ile Glu Ser Leu Gln Glu Leu Leu Arg Glu Gln Val Glu Asn

130

135

140

Tyr Tyr Ser Leu Pro Gly Gln Ser Cys Ser Glu Pro Thr Ser Pro Thr

145

150

155

160

Ser Asn Cys Ser Asp Gly Met Pro Glu Cys Asn Ser Pro Val Trp Ser

165

170

175

Arg Lys Ser Ser Thr Phe Asp Ser Ile Tyr Cys Pro Asp Val Ser Asn

180

185

190

Val Tyr Ala Thr Asp Lys Asn Ser Leu Ser Ser Leu Asp Cys Leu Ser

195

200

205

Asn Ile Val Asp Arg Ile Thr Ser Ser Glu Gln Pro Gly Leu Pro Leu

210

215

220

Gln Asp Leu Ala Ser Leu Ser Pro Val Ala Ser Thr Asp Ser Gln Pro

225

230

235

240

Arg Thr Pro Gly Ala Ser Ser Ser Arg Leu Ile Tyr His Val Leu

245

250

255

<210> 13

<211> 675

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (i) .. (675)

<400> 13

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Met Glu Leu Tyr Glu Thr Ser Pro Tyr Phe Tyr Gln Glu Pro Arg Phe

1

5

10

15

tat gat ggg gaa aac tac ctg cct gtc cac ctc cag gcc ttc gaa cca 96

Tyr Asp Gly Glu Asn Tyr Leu Pro Val His Leu Gln Gly Phe Glu Pro

20

25

30

cca gcc tac gag cgg acc gag ctc acc ctg agc ccc gag gcc cca ggg 144

Pro Gly Tyr Glu Arg Thr Glu Leu Thr Leu Ser Pro Glu Ala Pro Gly

35	40	45	
ccc ctt gag gac aag ggg ctg ggg acc ccc gag cac tgt cca ggc cag			192
Pro Leu Glu Asp Lys Gly Leu Gly Thr Pro Glu His Cys Pro Gly Gln			
50	55	60	
tgc ctg ccg tgg ggc tgt aag gtg tgt aag agc aag tcc gtg tcc gtg			240
Cys Leu Pro Trp Ala Cys Lys Val Cys Lys Arg Lys Ser Val Ser Val			
65	70	75	80
gac cgg cgg cgg ggc ggc aca ctg aag gag aag ggc agc ctc aag aag			288
Asp Arg Arg Arg Ala Ala Thr Leu Arg Glu Lys Arg Arg Leu Lys Lys			
85	90	95	
gtg aat gag ggc ttc gag gcc ctg aag aga agc acc ctg ctc aac ccc			336
Val Asn Glu Ala Phe Glu Ala Leu Lys Arg Ser Thr Leu Leu Asn Pro			
100	105	110	
aac cag cgg ctg ccc aag gtg gag atc ctg cgc agt ggc atc cag tac			384
Asn Gln Arg Leu Pro Lys Val Glu Ile Leu Arg Ser Ala Ile Gln Tyr			
115	120	125	
atc gag cgc ctc cag gcc ctg ctc agc tcc ctc aac cag gag gag cgt			432
Ile Glu Arg Leu Gln Ala Leu Leu Ser Ser Leu Asn Gln Glu Glu Arg			
130	135	140	
gac ctc cgc tac cgg ggc ggg ggc ggg ccc cag cca ggg gtg ccc agc			480
Asp Leu Arg Tyr Arg Gly Gly Gly Gly Pro Gln Pro Gly Val Pro Ser			
145	150	155	160
gaa tgc agc tct cac agc gcc tcc tgc agt cca gag tgg ggc agt gca			528
Glu Cys Ser Ser His Ser Ala Ser Cys Ser Pro Glu Trp Gly Ser Ala			
165	170	175	
ctg gag ttc agc gcc aac cca ggg gat cat ctg ctc aac gct gac cct			576
Leu Glu Phe Ser Ala Asn Pro Gly Asp His Leu Leu Thr Ala Asp Pro			

180	185	190	
acc ggt gcc cac aac ctg cac tcc ctg acc tcc atc gtg gac agc atc			624
Thr Asp Ala His Asn Leu His Ser Leu Thr Ser Ile Val Asp Ser Ile			
195	200	205	
acc gtg gaa gat gtg tct gtg gcc ttc cca gat gaa acc atg ccc aac			672
Thr Val Glu Asp Val Ser Val Ala Phe Pro Asp Glu Thr Met Pro Asn			
210	215	220	
tag			675

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<211> 224

<212> PRT

<213> Homo sapiens

<400> 14

Met	Glu	Leu	Tyr	Glu	Thr	Ser	Pro	Tyr	Phe	Tyr	Gln	Glu	Pro	Arg	Phe
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Tyr	Asp	Gly	Glu	Asn	Tyr	Leu	Pro	Val	His	Leu	Gln	Gly	Phe	Glu	Pro
	20					25							30		

Pro	Gly	Tyr	Glu	Arg	Thr	Glu	Leu	Thr	Leu	Ser	Pro	Glu	Ala	Pro	Gly
	35					40						45			

Pro	Leu	Glu	Asp	Lys	Gly	Leu	Gly	Thr	Pro	Glu	His	Cys	Pro	Gly	Gln
	50					55						60			

Cys Leu Pro Trp Ala Cys Lys Val Cys Lys Arg Lys Ser Val Ser Val
65 70 75 80

Asp Arg Arg Arg Ala Ala Thr Leu Arg Glu Lys Arg Arg Leu Lys Lys
85 90 95

Val Asn Glu Ala Phe Glu Ala Leu Lys Arg Ser Thr Leu Leu Asn Pro
100 105 110

Asn Gln Arg Leu Pro Lys Val Glu Ile Leu Arg Ser Ala Ile Gln Tyr
115 120 125

Ile Glu Arg Leu Gln Ala Leu Leu Ser Ser Leu Asn Gln Glu Glu Arg
130 135 140

Asp Leu Arg Tyr Arg Gly Gly Gly Gly Pro Gln Pro Gly Val Pro Ser
145 150 155 160

Glu Cys Ser Ser His Ser Ala Ser Cys Ser Pro Glu Trp Gly Ser Ala
165 170 175

Leu Glu Phe Ser Ala Asn Pro Gly Asp His Leu Leu Thr Ala Asp Pro
180 185 190

Thr Asp Ala His Asn Leu His Ser Leu Thr Ser Ile Val Asp Ser Ile
195 200 205

Thr Val Glu Asp Val Ser Val Ala Phe Pro Asp Glu Thr Met Pro Asn
 210 215 220

<210> 15

<211> 3935

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (373)..(1902)

<400> 15

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gtttcaaccc cggaaacttt tctttgcagg aggagagag aggggtgca agcgcaccca 120

ctttgtctct ttttctccc ctctctctcc tctcaattc gctcccccc acttggagcg 180

ggcagctgtg aactggcac cccgcgcctt cctcagtgct cgcggggta gcgggcgac 240

ggccagctt cccgggaga cgtttgctcc gcatccgag agccgaggg agaggagccc 300

ggcctcgag tcccgagcc gccgcgcctt ctgcctttc caggccacca gccccctgcc 360

ccggccccc gt atg aat ctc ctg gac ccc ttc atg aag atg acc gac gag 411

Met Asn Leu Leu Asp Pro Phe Met Lys Met Thr Asp Glu

1

5

10

cag gag aag ggc ctg tcc ggc gcc ccc agc ccc acc atg tcc gag gac 459

Gln Glu Lys Gly Leu Ser Gly Ala Pro Ser Pro Thr Met Ser Glu Asp

15

20

25

tcc gcg ggc tgg ccc tgc ccg tgg gcc tcc ggc tgg gac acc gag aac 507

Ser Ala Gly Ser Pro Cys Pro Ser Gly Ser Gly Ser Asp Thr Glu Asn
 30 35 40 45
 aag cag ccc cag gag aac acg ttc ccc aag ggc gag ccc gat ctg aag 555
 Thr Arg Pro Gln Glu Asn Thr Phe Pro Lys Gly Glu Pro Asp Leu Lys
 50 55 60
 aag gag agc gag gag gac aag ttc ccc gtg tgc atc cgc gag gcg gtc 603
 Lys Glu Ser Glu Glu Asp Lys Phe Pro Val Cys Ile Arg Glu Ala Val
 65 70 75
 agc cag atg ctg aac ggc tac gac tgg acg ctg gtc ccc atg ccg gtg 651
 Ser Gln Val Leu Lys Gly Tyr Asp Trp Thr Leu Val Pro Met Pro Val
 80 85 90
 cgc gtc aac ggc tac agc aag aac aag ccg cac gtc aag cag ccc atg 699
 Arg Val Asn Gly Ser Ser Lys Asn Lys Pro His Val Lys Arg Pro Met
 95 100 105
 aac ggc ttc atg gtg tgg gcg cag gcg gcg cgc aag aag ctg gcg gac 747
 Asn Ala Phe Met Val Trp Ala Gln Ala Ala Arg Arg Lys Leu Ala Asp
 110 115 120 125
 cag tac ccg ccc ttg cac aac gcc gag ctg agc aag acg ctg ggc aag 795
 Gln Tyr Pro His Leu His Asn Ala Glu Leu Ser Lys Thr Leu Gly Lys
 130 135 140
 ctg tgg aga ctt ctg aac gag agc gag aag ccg ccc ttc gtg gag gag 843
 Leu Trp Arg Leu Leu Asn Glu Ser Glu Lys Arg Pro Phe Val Glu Glu
 145 150 155
 gcc gag ccg ctg cgc gtg cag cac aag aag gac cac ccg gat tac aag 891
 Ala Glu Arg Leu Arg Val Gln His Lys Lys Asp His Pro Asp Tyr Lys
 160 165 170
 tac ccg ccg ccg ccg aag aag tgg gtg aag aac ggc cag gcg gag gca 939

Tyr Gln Pro Arg Arg Arg Lys Ser Val Lys Asn Gly Gln Ala Glu Ala
 175 180 185

gag gag gcc acg gag cag acg cac atc tcc ccc aac gcc atc ttc aag 987
 Glu Glu Ala Thr Glu Gln Thr His Ile Ser Pro Asn Ala Ile Phe Lys
 190 195 200 205

gca ctg cag gcc gac tgg cca cac tcc tcc tcc gcc atg agc gag gtg 1035
 Ala Leu Gln Ala Asp Ser Pro His Ser Ser Ser Gly Met Ser Glu Val
 210 215 220

cac tcc ccc gcc gag cac tgg ggg caa tcc cag gcc cca ccg acc cca 1083
 His Ser Pro Gly Glu His Ser Gly Gln Ser Gln Gly Pro Pro Thr Pro
 225 230 235

ccc acc acc ccc aac acc gac gtg cag ccg gcc aag gct gac ctg aag 1131
 Pro Thr Thr Pro Lys Thr Asp Val Gln Pro Gly Lys Ala Asp Leu Lys
 240 245 250

cea gag ggg cgc ccc ttg cca gag ggg gcc aga cag ccc cct atc gac 1179
 Arg Glu Gly Arg Pro Leu Pro Glu Gly Gly Arg Gln Pro Pro Ile Asp
 255 260 265

ttc cgc gac gtg gac atc gcc gag ctg agc agc gac gtc atc tcc aac 1227
 Phe Arg Asp Val Asp Ile Gly Glu Leu Ser Ser Asp Val Ile Ser Asn
 270 275 280 285

atc gag acc ttc gat gtc aac gag ttt gac cag tac ctg ccg ccc aac 1275
 Ile Glu Thr Phe Asp Val Asn Glu Phe Asp Gln Tyr Leu Pro Pro Asn
 290 295 300

gcc cac ccg ggg gtg ccg gcc acg cac gcc cag gtc acc tac acc gcc 1323
 Gly His Pro Gly Val Pro Ala Thr His Gly Gln Val Thr Tyr Thr Gly
 305 310 315

agc tac gcc atc agc agc acc gcg gcc acc ccg gcc agc gcc gcc cac 1371

Ser Tyr Gly Ile Ser Ser Thr Ala Ala Thr Pro Ala Ser Ala Gly His
 320 325 330

gtg tgg atg tcc aag cag cag gcg cgg ccg cca ccc ccg cag cag ccc 1419
 Val Trp Met Ser Lys Gln Gln Ala Pro Pro Pro Pro Pro Gln Gln Pro
 335 340 345

cca cag gcc cgg ccg gcc ccg cag gcg ccc ccg cag ccg cag gcc gcg 1467
 Pro Gln Ala Pro Pro Ala Pro Gln Ala Pro Pro Gln Pro Gln Ala Ala
 350 355 360 365

ccc cca cag cag ccg gcg gcc ccc ccg cag cag cca cag gcc cag acc 1515
 Pro Pro Gln Gln Pro Ala Ala Pro Pro Gln Gln Pro Gln Ala His Thr
 370 375 380

ctg acc acc ctg agc agc gag ccg gcc cag tcc cag cga acc cag atc 1563
 Leu Thr Thr Leu Ser Ser Glu Pro Gly Gln Ser Gln Arg Thr His Ile
 385 390 395

aag acc gag cag ctg agc ccc agc cag tac agc gag cag cag cag cag 1611
 Lys Thr Glu Gln Leu Ser Pro Ser His Tyr Ser Glu Gln Gln Gln His
 400 405 410

tgg ccc cca cag atc gcc tac agc ccc ttc aac ctc cca cag tac agc 1659
 Ser Pro Gln Gln Ile Ala Tyr Ser Pro Phe Asn Leu Pro His Tyr Ser
 415 420 425

ccc tcc tac ccg ccc atc acc cgc tca cag tac gcc tac acc gcc cag 1707
 Pro Ser Tyr Pro Pro Ile Thr Arg Ser Gln Tyr Asp Tyr Thr Asp His
 430 435 440 445

cag aac tcc agc tcc tac tcc agc cag gcc gcc gcc cag gcc acc gcc 1755
 Gln Asn Ser Ser Ser Tyr Tyr Ser His Ala Ala Gly Gln Gly Thr Gly
 450 455 460

ctc tac tcc acc ttc acc tac atg aac ccc gct cag cgc ccc atg tac 1803

Leu Tyr Ser Thr Phe Thr Tyr Met Asn Pro Ala Gln Arg Pro Met Tyr
 465 470 475

acc ccc atc gcc gac acc tct ggg gtc cct tcc atc ccc cag acc cac 1851
 Thr Pro Ile Ala Asp Thr Ser Gly Val Pro Ser Ile Pro Gln Thr His
 480 485 490

agg ccc cag cac tgg gaa gaa ccc gtc tac aca cag ctc act cga cct 1899
 Ser Pro Gln His Trp Glu Gln Pro Val Tyr Thr Gln Leu Thr Arg Pro
 495 500 505

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cgaagaaaga gaggacccac cagaattccc ttggacatt tgtgtttttt tgtttttta 2012

ttttgttttg tttttcttc tttttctct tcttaaaaga catttatgct aaaggcaact 2072

cgtacccaaa ttcccaagac acaaacatga cctatccaag cgcattaccc acttgigccc 2132

actcagtggo caggccaacc ttggctaast ggagcagcga aatcaatcag aaaciggact 2192

tttaaaccc tcttcagacn aagcctggcg gatgatggag actcgtgiga tcagtggtct 2252

aaatctctct gctgttttg actttgtaat tatttttta gcagtaatta aagaaaaag 2312

tctctgtga ggaatattct ctattttaaa tattttiagt atgtactgtg tatgatccat 2372

taccatttg aggggattha tacatatatt tagataaast taatgctct tatttttcca 2432

acagctaac tctctiagt tgaacagtgt gccctagctt ttcttgcaac cagagtattt 2492

tgtacagat ttgttttctc ttacaaaaag aaaaaaaa tctgtttgta ttaacattta 2552

aaacagaa! tgtgtatgt gatcagtttt ggaagttaac ttgccttaast tctcaggt 2612

tgcgattha agggggagct gccttaaaa aaataaagg ccttatttg caattatgg 2672

agtasacaat agtctagaga agcatttggi agcttttiao etatatatat tttttasaga 2732

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gcatttcctc ctgcctttag ttgtttacgt cagtcttaag aagaggtaa aaggcaagca 2852

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tgaaggaga ggttttaatt aaacaaaaa aaattcttt tttttttt ttccaattt 3092

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taaaagcaag ttcttttga ttctcaccc tagatttga taatgcctt ttgtccctc 3272

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cagttttaga agtcagtag aiaaaactt aaagcactca taatagga taattcaatt 3692

tatgtatasa agcagactt tttaaaaa tacttttga acttaagaaa ctggcattt 3752

aaatcatatt ttgtatttag gtaaaagott tggitttgt tegtgttttg ttgttttca 3812
 ttgttttcaat ccaagcccca aacatttgt tctctcgtg aaacttacct ttccattttt 3872
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 aaa 3935

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 <212> PRT
 <213> Homo sapiens

<400> 16

Met Asn Leu Leu Asp Pro Phe Met Lys Met Thr Asp Glu Gln Glu Lys
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Gly Leu Ser Gly Ala Pro Ser Pro Thr Met Ser Glu Asp Ser Ala Gly
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Ser Pro Cys Pro Ser Gly Ser Gly Ser Asp Thr Glu Asn Thr Arg Pro
 35 40 45

Gln Glu Asn Thr Phe Pro Lys Gly Glu Pro Asp Leu Lys Lys Glu Ser
 50 55 60

Glu Glu Asp Lys Phe Pro Val Cys Ile Arg Glu Ala Val Ser Gln Val
 65 70 75 80

Leu Lys Gly Tyr Asp Trp Thr Leu Val Pro Met Pro Val Arg Val Asn
85 90 95

Gly Ser Ser Lys Asn Lys Pro His Val Lys Arg Pro Met Asn Ala Phe
100 105 110

Met Val Trp Ala Gln Ala Ala Arg Arg Lys Leu Ala Asp Gln Tyr Pro
115 120 125

His Leu His Asn Ala Glu Leu Ser Lys Thr Leu Gly Lys Leu Trp Arg
130 135 140

Leu Leu Asn Glu Ser Glu Lys Arg Pro Phe Val Glu Glu Ala Glu Arg
145 150 155 160

Leu Arg Val Gln His Lys Lys Asp His Pro Asp Tyr Lys Tyr Gln Pro
165 170 175

Arg Arg Arg Lys Ser Val Lys Asn Gly Gln Ala Glu Ala Glu Glu Ala
180 185 190

Thr Glu Gln Thr His Ile Ser Pro Asn Ala Ile Phe Lys Ala Leu Gln
195 200 205

Ala Asp Ser Pro His Ser Ser Ser Gly Met Ser Glu Val His Ser Pro
210 215 220

Gly Glu His Ser Gly Gln Ser Gln Gly Pro Pro Thr Pro Pro Thr Thr
225 230 235 240

Pro Lys Thr Asp Val Gln Pro Gly Lys Ala Asp Leu Lys Arg Glu Gly
245 250 255

Arg Pro Leu Pro Glu Gly Gly Arg Gln Pro Pro Ile Asp Phe Arg Asp
260 265 270

Val Asp Ile Gly Glu Leu Ser Ser Asp Val Ile Ser Asn Ile Glu Thr
275 280 285

Phe Asp Val Asn Glu Phe Asp Gln Tyr Leu Pro Pro Asn Gly His Pro
290 295 300

Gly Val Pro Ala Thr His Gly Gln Val Thr Tyr Thr Gly Ser Tyr Gly
305 310 315 320

Ile Ser Ser Thr Ala Ala Thr Pro Ala Ser Ala Gly His Val Trp Met
325 330 335

Ser Lys Gln Gln Ala Pro Pro Pro Pro Pro Gln Gln Pro Pro Gln Ala
340 345 350

Pro Pro Ala Pro Gln Ala Pro Pro Gln Pro Gln Ala Ala Pro Pro Gln
355 360 365

Gln Pro Ala Ala Pro Pro Gln Gln Pro Gln Ala His Thr Leu Thr Thr
370 375 380

Leu Ser Ser Glu Pro Gly Gln Ser Gln Arg Thr His Ile Lys Thr Glu
385 390 395 400

Gln Leu Ser Pro Ser His Tyr Ser Glu Gln Gln Gln His Ser Pro Gln
405 410 415

Gln Ile Ala Tyr Ser Pro Phe Asn Leu Pro His Tyr Ser Pro Ser Tyr
420 425 430

Pro Pro Ile Thr Arg Ser Gln Tyr Asp Tyr Thr Asp His Gln Asn Ser
435 440 445

Ser Ser Tyr Tyr Ser His Ala Ala Gly Gln Gly Thr Gly Leu Tyr Ser
450 455 460

Thr Phe Thr Tyr Met Asn Pro Ala Gln Arg Pro Met Tyr Thr Pro Ile
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Ala Asp Thr Ser Gly Val Pro Ser Ile Pro Gln Thr His Ser Pro Gln
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His Trp Glu Gln Pro Val Tyr Thr Gln Leu Thr Arg Pro
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<210> 17
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<220>
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 <222> (158)..(4621)

<400> 17

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cgcccgagcc cggctcagcc aggcgcgcgc gtgagcc atg att cgc ctg ggg gct 175

Met Ile Arg Leu Gly Ala

1

5

ccc cag tgg ctg gtg ctg ctg aag ctg ctg gtc gcc gat gtc att cgg 223

Pro Gln Ser Leu Val Leu Leu Thr Leu Leu Val Ala Ala Val Leu Arg

10

15

20

tgt cag gcc cag gat gtc cag gag gat gcc agc tgt gtg cag gat ggg 271

Cys Gln Gly Gln Asp Val Gln Glu Ala Gly Ser Cys Val Gln Asp Gly

25

30

35

cag agg tat aat gat aag gat gtg tgg aag ccg gag ccc tgc cgg etc 319

Gln Arg Tyr Asn Asp Lys Asp Val Trp Lys Pro Glu Pro Cys Arg Ile

40

45

50

tgt gtc tgt gac act ggg act gtc ctg tgc gac gac ata atc tgt gaa 367

Cys Val Cys Asp Thr Gly Thr Val Leu Cys Asp Asp Ile Ile Cys Glu

65

60

65

70

gac gtc aaa gac tgc ctc agc cat gag atc ccc ttc gga sag tgc tgc	415
Asp Val Lys Asp Cys Leu Ser Pro Glu Ile Pro Phe Gly Glu Cys Cys	
75 80 85	
ccc atc tgc cca act gac ctc gcc act gcc agt ggg cca cca gga cca	463
Pro Ile Cys Pro Thr Asp Leu Ala Thr Ala Ser Gly Gln Pro Gly Pro	
90 95 100	
aag gga cag aaa gga gaa cct gga gac atc aag gat att gta gga ccc	511
Lys Gly Gln Lys Gly Glu Pro Gly Asp Ile Lys Asp Ile Val Gly Pro	
105 110 115	
aaa gga cct cct ggg cct cag gga cct gaa ggg gaa cca gga ccc aga	559
Lys Gly Pro Pro Gly Pro Gln Gly Pro Ala Gly Glu Gln Gly Pro Arg	
120 125 130	
ggg gat cgt ggt gac aaa ggt gaa aaa ggt gcc cct gga cct cgt gcc	607
Gly Asp Arg Gly Asp Lys Gly Glu Lys Gly Ala Pro Gly Pro Arg Gly	
135 140 145 150	
aga gat gga gaa cct ggg acc cct gga aat cct gcc ccc cct ggt cct	655
Arg Asp Gly Glu Pro Gly Thr Pro Gly Asn Pro Gly Pro Pro Gly Pro	
155 160 165	
ccc gcc ccc cct ggt ccc cct ggt ctt ggt gga aac ttt gct gcc cag	703
Pro Gly Pro Pro Gly Pro Pro Gly Leu Gly Gly Asn Phe Ala Ala Gln	
170 175 180	
atg gct gga gga ttt gat gaa aag gct ggt ggc gcc cag ttg gga gta	751
Met Ala Gly Gly Phe Asp Glu Lys Ala Gly Gly Ala Gln Leu Gly Val	
185 190 195	
atg caa gga cca atg gcc ccc atg gga cct cga gga cct cca gcc cct	799
Met Gln Gly Pro Met Gly Pro Met Gly Pro Arg Gly Pro Pro Gly Pro	
200 205 210	

gca ggt gct cct ggg cct caa gga ttt caa ggc aat cct ggt gaa cct 847
 Ala Gly Ala Pro Gly Pro Gln Gly Phe Gln Gly Asn Pro Gly Glu Pro
 215 220 225 230

ggt gaa cct ggt gtc tct ggt ccc atg ggt ccc cgt ggt cct cct ggt 895
 Gly Glu Pro Gly Val Ser Gly Pro Met Gly Pro Arg Gly Pro Pro Gly
 235 240 245

ccc cct gga aag cct ggt gat gat ggt gaa gct gga aaa cct gga aaa 943
 Pro Pro Gly Lys Pro Gly Asp Asp Gly Glu Ala Gly Lys Pro Gly Lys
 250 255 260

ggt ggt gaa agg ggt ccc cct ggt cct cag ggt gct cgt ggt ttc cca 991
 Ala Gly Glu Arg Gly Pro Pro Gly Pro Gln Gly Ala Arg Gly Phe Pro
 265 270 275

gga aac cca ggc ctt cct ggt gtc aaa ggt cac aga ggt tat cca ggc 1039
 Gly Thr Pro Gly Leu Pro Gly Val Lys Gly His Arg Gly Tyr Pro Gly
 280 285 290

ctg gac ggt gct aag gga gag gcg ggt gct cct ggt gta aag ggt gag 1087
 Leu Asp Gly Ala Lys Gly Glu Ala Gly Ala Pro Gly Val Lys Gly Glu
 295 300 305 310

agt ggt tcc ccg ggt gag aac gga tct ccg ggc cca atg ggt cct cgt 1135
 Ser Gly Ser Pro Gly Glu Asn Gly Ser Pro Gly Pro Met Gly Pro Arg
 315 320 325

ggc ctg cct ggt gaa aga gga ccg act ggc cct gct ggc gct gcg ggt 1183
 Gly Leu Pro Gly Glu Arg Gly Arg Thr Gly Pro Ala Gly Ala Ala Gly
 330 335 340

ggc cga ggc aac gat ggt cag cca ggc ccc gca ggt cct ccg ggt cct 1231
 Ala Arg Gly Asn Asp Gly Gln Pro Gly Pro Ala Gly Pro Pro Gly Pro
 345 350 355

gtc ggt cct gct ggt ggt cct ggc ttc cct ggt gct cct gga ggc aag	1279
Val Gly Pro Ala Gly Gly Pro Gly Phe Pro Gly Ala Pro Gly Ala Lys	
360 365 370	
ggt gaa ggc ggc ccc act ggt gcc cgt ggt cct gaa ggt gct caa ggt	1327
Gly Glu Ala Gly Pro Thr Gly Ala Arg Gly Pro Glu Gly Ala Gln Gly	
375 380 385 390	
cct cgc ggt gaa cct ggt act cct ggg tcc cct ggg cct gct ggt gcc	1375
Pro Arg Gly Glu Pro Gly Thr Pro Gly Ser Pro Gly Pro Ala Gly Ala	
395 400 405	
tcc ggt aac cct gga aca gat gga att cct gga gcc aac gga tct gct	1423
Ser Gly Asn Pro Gly Thr Asp Gly Ile Pro Gly Ala Lys Gly Ser Ala	
410 415 420	
ggt gct cct ggc att gct ggt gct cct ggc ttc cct ggg cca cgg ggt	1471
Gly Ala Pro Gly Ile Ala Gly Ala Pro Gly Phe Pro Gly Pro Arg Gly	
425 430 435	
cct cct ggc cct caa ggt gca act ggt cct ctg ggc ccg aaa ggt cag	1519
Pro Pro Gly Pro Gln Gly Ala Thr Gly Pro Leu Gly Pro Lys Gly Gln	
440 445 450	
acg ggt gaa cct ggt att gct ggc ttc aaa ggt gaa caa ggc ccc aag	1567
Thr Gly Glu Pro Gly Ile Ala Gly Phe Lys Gly Glu Gln Gly Pro Lys	
455 460 465 470	
gga gaa cct ggc cct gct ggc ccc cag gga gcc cct gga ccc gct ggt	1615
Gly Glu Pro Gly Pro Ala Gly Pro Gln Gly Ala Pro Gly Pro Ala Gly	
475 480 485	
gaa gaa ggc aag aga ggt gcc cgt gga gag cct ggt ggc att ggc ccc	1663
Glu Glu Gly Lys Arg Gly Ala Arg Gly Glu Pro Gly Gly Val Gly Pro	
490 495 500	

atc ggt ccc cct gga gaa aga ggt ggt ccc gga aac cgc ggt ttc cca 1711
 lle Gly Pro Pro Gly Glu Arg Gly Ala Pro Gly Asn Arg Gly Phe Pro
 505 510 515

ggt caa gat ggt ctg gca ggt ccc aag gga gcc cct gga gag cga gga 1759
 Gly Gln Asp Gly Leu Ala Gly Pro Lys Gly Ala Pro Gly Glu Arg Gly
 520 525 530

ccc agt ggt ctt gct ggc ccc aag gga gcc aac ggt gac cct ggc cgt 1807
 Pro Ser Gly Leu Ala Gly Pro Lys Gly Ala Asn Gly Asp Pro Gly Arg
 535 540 545 550

cct gga gaa cct ggc ctt cct gga gcc cgg ggt ctc act ggc cgc cct 1855
 Pro Gly Glu Pro Gly Leu Pro Gly Ala Arg Gly Leu Thr Gly Arg Pro
 555 560 565

ggt gat gct ggt cct caa gcc aaa gtt ggc cct tct gga gcc cct ggt 1903
 Gly Asp Ala Gly Pro Gln Gly Lys Val Gly Pro Ser Gly Ala Pro Gly
 570 575 580

gaa gat ggt cgt cct gga cct cca ggt cct cag gga gct cgt gga cag 1951
 Glu Asp Gly Arg Pro Gly Pro Pro Gly Pro Gln Gly Ala Arg Gly Gln
 585 590 595

cct ggt gtc atg ggt ttc cct ggc ccc aaa ggt gcc aac ggt gag cct 1999
 Pro Gly Val Met Gly Phe Pro Gly Pro Lys Gly Ala Asn Gly Glu Pro
 600 605 610

ggc aaa gct ggt gag aag gga ctg cct ggt gct cct ggt ctg agg ggt 2047
 Gly Lys Ala Gly Glu Lys Gly Leu Pro Gly Ala Pro Gly Leu Arg Gly
 615 620 625 630

ctt cct ggc aaa gat ggt gag aca ggt gct gca gga ccc cct ggc cct 2095
 Leu Pro Gly Lys Asp Gly Glu Thr Gly Ala Ala Gly Pro Pro Gly Pro
 635 640 645

gct gga cct gct ggt gaa cga ggc gag cag ggt gct cct ggc cca tct 2143
 Ala Gly Pro Ala Gly Glu Arg Gly Glu Gln Gly Ala Pro Gly Pro Ser
 650 655 660

ggg ttc cag gga ctt cct ggc cct cct ggt ccc cca ggt gaa ggt gga 2191
 Gly Phe Gln Gly Leu Pro Gly Pro Pro Gly Pro Pro Gly Glu Gly Gly
 665 670 675

aaa cca ggt gac cag ggt gtt ccc ggt gaa gct gga gcc cct ggc ctc 2239
 Lys Pro Gly Asp Gln Gly Val Pro Gly Glu Ala Gly Ala Pro Gly Leu
 680 685 690

gig ggt ccc agg ggt gaa cga ggt ttc cca ggt gaa cgt ggc tct ccc 2287
 Val Gly Pro Arg Gly Glu Arg Gly Phe Pro Gly Glu Arg Gly Ser Pro
 695 700 705 710

ggt gcc cag ggc ctc cag ggt ccc cgt ggc ctc ccc ggc act cct ggc 2335
 Gly Ala Gln Gly Leu Gln Gly Pro Arg Gly Leu Pro Gly Thr Pro Gly
 715 720 725

act ggt ggt ccc aaa ggt gca tct ggc cca gca ggc ccc cct ggc gca 2383
 Thr Asp Gly Pro Lys Gly Ala Ser Gly Pro Ala Gly Pro Pro Gly Ala
 730 735 740

cag ggc cct cca ggt ctt cag gga atg cct ggc gag agg gga gca gct 2431
 Gln Gly Pro Pro Gly Leu Gln Gly Met Pro Gly Glu Arg Gly Ala Ala
 745 750 755

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 Gly Ile Ala Gly Pro Lys Gly Asp Arg Gly Asp Val Gly Glu Lys Gly
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cct gag gga gcc cct gga aag gat ggt gga cga ggc ctc aca ggt ccc 2527
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 ile Gly Pro Pro Gly Pro Ala Gly Ala Asn Gly Glu Lys Gly Glu Val
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gca cct ggg cct cag ggt cct act gga gta cct ggt cct aaa gga gcc 2815
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30

Ser Cys Val Gln Asp Gly Gln Arg Tyr Asn Asp Lys Asp Val Trp Lys

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40

45

Pro Glu Pro Cys Arg Ile Cys Val Cys Asp Thr Gly Thr Val Leu Cys
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Asp Asp Ile Ile Cys Glu Asp Val Lys Asp Cys Leu Ser Pro Glu Ile
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Pro Phe Gly Glu Cys Cys Pro Ile Cys Pro Thr Asp Leu Ala Thr Ala
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Ser Gly Gln Pro Gly Pro Lys Gly Gln Lys Gly Glu Pro Gly Asp Ile
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Lys Asp Ile Val Gly Pro Lys Gly Pro Pro Gly Pro Gln Gly Pro Ala
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Ala Pro Gly Pro Arg Gly Arg Asp Gly Glu Pro Gly Thr Pro Gly Asn
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Pro Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly Leu Gly
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Gly Asn Phe Ala Ala Gln Met Ala Gly Gly Phe Asp Glu Lys Ala Gly
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Arg Gly Pro Pro Gly Pro Ala Gly Ala Pro Gly Pro Gln Gly Phe Gln
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Gly Asn Pro Gly Glu Pro Gly Glu Pro Gly Val Ser Gly Pro Met Gly
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Pro Arg Gly Pro Pro Gly Pro Pro Gly Lys Pro Gly Asp Asp Gly Glu
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Ala Gly Lys Pro Gly Lys Ala Gly Glu Arg Gly Pro Pro Gly Pro Gln
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Gly Ala Arg Gly Phe Pro Gly Thr Pro Gly Leu Pro Gly Val Lys Gly
275 280 285

His Arg Gly Tyr Pro Gly Leu Asp Gly Ala Lys Gly Glu Ala Gly Ala
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Pro Gly Val Lys Gly Glu Ser Gly Ser Pro Gly Glu Asn Gly Ser Pro
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Gly Pro Met Gly Pro Arg Gly Leu Pro Gly Glu Arg Gly Arg Thr Gly
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Ala Gly Pro Pro Gly Pro Val Gly Pro Ala Gly Gly Pro Gly Phe Pro
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Pro Gly Pro Ala Gly Ala Ser Gly Asn Pro Gly Thr Asp Gly Ile Pro
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Gly Ala Lys Gly Ser Ala Gly Ala Pro Gly Ile Ala Gly Ala Pro Gly
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Phe Pro Gly Pro Arg Gly Pro Pro Gly Pro Gln Gly Ala Thr Gly Pro
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Leu Gly Pro Lys Gly Gln Thr Gly Glu Pro Gly Ile Ala Gly Phe Lys
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Gln Gly Ala Arg Gly Gln Pro Gly Val Met Gly Phe Pro Gly Pro Lys
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Ala Pro Gly Leu Arg Gly Leu Pro Gly Lys Asp Gly Glu Thr Gly Ala
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Pro Pro Gly Glu Gly Gly Lys Pro Gly Asp Gln Gly Val Pro Gly Glu
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Leu Pro Gly Thr Pro Gly Thr Asp Gly Pro Lys Gly Ala Ser Gly Pro
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Ala Gly Pro Pro Gly Ala Gln Gly Pro Pro Gly Leu Gln Gly Met Pro
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755 760 765

Asp Val Gly Glu Lys Gly Pro Glu Gly Ala Pro Gly Lys Asp Gly Gly
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Arg Gly Leu Thr Gly Pro Ile Gly Pro Pro Gly Pro Ala Gly Ala Asn
785 790 795 800

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850 855 860

Pro Gln Gly Pro Ser Gly Ala Pro Gly Pro Gln Gly Pro Thr Gly Val
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Thr Gly Pro Lys Gly Ala Arg Gly Ala Gln Gly Pro Pro Gly Ala Thr
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Gly Phe Pro Gly Ala Ala Gly Arg Val Gly Pro Pro Gly Ser Asn Gly
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Asn Pro Gly Pro Pro Gly Pro Pro Gly Pro Ser Gly Lys Asp Gly Pro
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Lys Gly Ala Arg Gly Asp Ser Gly Pro Pro Gly Arg Ala Gly Glu Pro
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Gly Leu Gln Gly Pro Ala Gly Pro Pro Gly Glu Lys Gly Glu Pro Gly
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Ile Pro Gln Pro Ser Pro Leu Arg Val Leu Leu Gly Thr Ser Leu Thr

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 Thr Tyr Asp Val Tyr Cys Phe Ala Glu Glu Met Glu Gly Glu Val Phe
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Trp Leu Ala Asp Gly Ser Leu Arg Tyr Pro Ile Val Thr Pro Arg Pro
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 Ala Cys Gly Gly Asp Lys Pro Gly Val Arg Thr Val Tyr Leu Tyr Pro
 645 650 655

 aac cag aag gcc ctg cca gac cca ctg tcc agg cag cat gcc ttc tgc 2076
 Asn Gln Thr Gly Leu Pro Asp Pro Leu Ser Arg His His Ala Phe Cys
 660 665 670

 ttc cga gcc att tca gcc gtt cct tct cca gaa gaa gaa gag ggt gcc 2124
 Phe Arg Gly Ile Ser Ala Val Pro Ser Pro Gly Glu Glu Glu Gly Gly
 675 680 685

 aca ccc aca tca ccc tct ggt gtg gag gag tgg atc gtg acc cca gtg 2172
 Thr Pro Thr Ser Pro Ser Gly Val Glu Glu Trp Ile Val Thr Gln Val
 690 695 700

 gtt cct ggt gtg gct gct gtc ccc gta gaa gag gag aca act gct gta 2220
 Val Pro Gly Val Ala Ala Val Pro Val Glu Glu Glu Thr Thr Ala Val
 705 710 715 720

 ccc tca ggg gag act act gcc atc cta gag ttc acc acc gag cca gaa 2268
 Pro Ser Gly Glu Thr Thr Ala Ile Leu Glu Phe Thr Thr Glu Pro Glu
 725 730 735

 aac cag aca gaa tgg gaa cca gcc tat acc cca gtg gcc aca tcc ccg 2316
 Asn Gln Thr Glu Trp Glu Pro Ala Tyr Thr Pro Val Gly Thr Ser Pro
 740 745 750

 ctg cca ggg atc att cct act tgg cct cct act gcc gcc gaa aca gag 2364
 Leu Pro Gly Ile Leu Pro Thr Trp Pro Pro Thr Gly Ala Glu Thr Glu
 755 760 765

 gaa agt aca gaa gcc cct tct gaa act gaa gtg ccc tct gcc tca gag 2412

Glu Ser Thr Glu Gly Pro Ser Ala Thr Glu Val Pro Ser Ala Ser Glu
 770 775 780

gaa cca tcc ccc tca gag gtg cca ttc ccc tca gag gag cca tcc ccc 2460
 Glu Pro Ser Pro Ser Glu Val Pro Phe Pro Ser Glu Glu Pro Ser Pro
 785 790 795 800

tca gag gaa cca ttc ccc tca gtg agg cca ttc ccc tca gtg gag ctg 2508
 Ser Glu Glu Pro Phe Pro Ser Val Arg Pro Phe Pro Ser Val Glu Leu
 805 810 815

ttc ccc tca gag gag cca ttc ccc tcc aag gag cca tcc ccc tca gag 2556
 Phe Pro Ser Glu Glu Pro Phe Pro Ser Lys Glu Pro Ser Pro Ser Glu
 820 825 830

gaa cca tca gcc tca gaa gag ccc tat cca cct tca ccc ccc gag ccc 2604
 Glu Pro Ser Ala Ser Glu Glu Pro Tyr Thr Pro Ser Pro Pro Glu Pro
 835 840 845

agc tgg act gag ctg ccc agc tct ggg gag gaa tct ggg gcc cct gat 2652
 Ser Trp Thr Glu Leu Pro Ser Ser Gly Glu Glu Ser Gly Ala Pro Asp
 850 855 860

gtc agt ggt gac ttc aca gcc agt gga gat gtt tca gga ccc ctt gac 2700
 Val Ser Gly Asp Phe Thr Gly Ser Gly Asp Val Ser Gly His Leu Asp
 865 870 875 880

ttc agt ggg cag ctg tca ggg gac agg gcc agt gga ctg ccc tct gga 2748
 Phe Ser Gly Gln Leu Ser Gly Asp Arg Ala Ser Gly Leu Pro Ser Gly
 885 890 895

gac ctg gac tcc agt ggt ctt act tcc aca ggg gcc tca gcc ctg act 2796
 Asp Leu Asp Ser Ser Gly Leu Thr Ser Thr Val Gly Ser Gly Leu Thr
 900 905 910

gtg gaa agt gga cta ccc tca ggg gat gaa gag aga att gag tgg ccc 2844

Val Glu Ser Gly Leu Pro Ser Gly Asp Glu Glu Arg Ile Glu Trp Pro
 915 920 925

agc acc cct acg gtt ggt gaa ctg ccc tct gga gct gag atc cta gag 2992
 Ser Thr Pro Thr Val Gly Glu Leu Pro Ser Gly Ala Glu Ile Leu Glu
 930 935 940

gag tct gcc tct gga gtt ggg gat ctc agt gga ctt cct tct gga gaa 2940
 Gly Ser Ala Ser Gly Val Gly Asp Leu Ser Gly Leu Pro Ser Gly Glu
 945 950 955 960

gtt cta gag acc tct gcc tct gga gta gga gac ctc agt gga ctt cct 2988
 Val Leu Glu Thr Ser Ala Ser Gly Val Gly Asp Leu Ser Gly Leu Pro
 965 970 975

tct gga gaa gtt cta gag acc act gcc cct gga gta gag gac atc agc 3036
 Ser Gly Glu Val Leu Glu Thr Thr Ala Pro Gly Val Glu Asp Ile Ser
 980 985 990

gag ctt cct tct gga gaa gtt cta gag acc act gcc cct gga gta gag 3084
 Gly Leu Pro Ser Gly Glu Val Leu Glu Thr Thr Ala Pro Gly Val Glu
 995 1000 1005

gag atc agc ggg ctt cct tct gga gaa gtt cta gag acc act gcc 3129
 Asp Ile Ser Gly Leu Pro Ser Gly Glu Val Leu Glu Thr Thr Ala
 1010 1015 1020

cct gga gta gag gac atc agc ggg ctt cct tct gga gaa gtt cta 3174
 Pro Gly Val Glu Asp Ile Ser Gly Leu Pro Ser Gly Glu Val Leu
 1025 1030 1035

gag acc act gcc cct gga gta gag gac atc agc ggg ctt cct tct 3219
 Glu Thr Thr Ala Pro Gly Val Glu Asp Ile Ser Gly Leu Pro Ser
 1040 1045 1050

gag gaa gtt cta gag acc act gcc cct gga gta gag gac atc agc 3264

Gly Glu	Val Leu Glu Thr Thr	Ala Pro Gly Val Glu	Asp Ile Ser	
1055	1060	1065		
ggg ctt	oct tct gga gaa gtt	cta gag acc gct gcc	cct gga gta	3309
Gly Leu	Pro Ser Gly Glu Val	Leu Glu Thr Ala Ala	Pro Gly Val	
1070	1075	1080		
ggg gac	atc agc ggg ctt oct	tct gga gaa gtt cta	gag acc gct	3354
Glu Asp	Ile Ser Gly Leu Pro	Ser Gly Glu Val Leu	Glu Thr Ala	
1085	1090	1095		
gac oct	ggg gta gag gac atc	agc ggg ctt oct tct	gga gaa gtt	3399
Ala Pro	Gly Val Glu Asp Ile	Ser Gly Leu Pro Ser	Gly Glu Val	
1100	1105	1110		
cta gag	acc gct gcc cct gga	gta gag gac atc agc	ggg ctt oct	3444
Leu Glu	Thr Ala Ala Pro Gly	Val Glu Asp Ile Ser	Gly Leu Pro	
1115	1120	1125		
tct gga	gaa gtt cta gag acc	gct gcc cct gga gta	gag gac atc	3489
Ser Gly	Glu Val Leu Glu Thr	Ala Ala Pro Gly Val	Glu Asp Ile	
1130	1135	1140		
agc ggg	ctt oct tct gga gaa	gtt cta gag acc gct	gcc oct gga	3534
Ser Gly	Leu Pro Ser Gly Glu	Val Leu Glu Thr Ala	Ala Pro Gly	
1145	1150	1155		
gta gag	gac atc agc ggg ctt	cct tct gga gaa gtt	cta gag acc	3579
Val Glu	Asp Ile Ser Gly Leu	Pro Ser Gly Glu Val	Leu Glu Thr	
1160	1165	1170		
gct gcc	cct gga gta gag gac	atc agc ggg ctt oct	tct gga gaa	3624
Ala Ala	Pro Gly Val Glu Asp	Ile Ser Gly Leu Pro	Ser Gly Glu	
1175	1180	1185		
gtt cta	gag acc gct gcc cct	gga gta gag gac atc	agc ggg ctt	3669

Val Leu	Glu Thr Ala Ala Pro	Gly Val Glu Asp Ile	Ser Gly Leu	
1190	1195	1200		
cct tct	gga gaa gtt cta gag	act gct gcc cct gga	gta gag gac	3714
Pro Ser	Gly Glu Val Leu Glu	Thr Ala Ala Pro Gly	Val Glu Asp	
1205	1210	1215		
atc agc	ggg ctt cct tct gga	gaa gtt cta gag act	gct gcc cct	3759
Ile Ser	Gly Leu Pro Ser Gly	Glu Val Leu Glu Thr	Ala Ala Pro	
1220	1225	1230		
gga gta	gag gac atc agc ggg	ctt cct tct gga gaa	gtt cta gag	3804
Gly Val	Glu Asp Ile Ser Gly	Leu Pro Ser Gly Glu	Val Leu Glu	
1235	1240	1245		
act gct	gcc cct gga gta gag	gac atc agc ggg ctt	cct tct gga	3849
Thr Ala	Ala Pro Gly Val Glu	Asp Ile Ser Gly Leu	Pro Ser Gly	
1250	1255	1260		
gaa gtt	cta gag act gct gcc	cct gga gta gag gac	atc agc ggg	3894
Glu Val	Leu Glu Thr Ala Ala	Pro Gly Val Glu Asp	Ile Ser Gly	
1265	1270	1275		
ctt cct	tct gga gaa gtt cta	gag act act gcc cct	gga gta gag	3939
Leu Pro	Ser Gly Glu Val Leu	Glu Thr Thr Ala Pro	Gly Val Glu	
1280	1285	1290		
gag atc	agc ggg ctt cct tct	gga gaa gtt cta gag	act act gcc	3984
Glu Ile	Ser Gly Leu Pro Ser	Gly Glu Val Leu Glu	Thr Thr Ala	
1295	1300	1305		
cct gga	gta gat gag atc agt	ggg ctt cct tct gga	gaa gtt cta	4029
Pro Gly	Val Asp Glu Ile Ser	Gly Leu Pro Ser Gly	Glu Val Leu	
1310	1315	1320		
gag act	act gcc cct gga gta	gag gag atc agc ggg	ctt cct tct	4074

Glu Thr	Thr Ala Pro Gly Val	Glu Glu Ile Ser Gly	Leu Pro Ser	
1325	1330	1335		
gga gaa	gtt cta gag aat tot	acc tot gcg gta ggg	gac ctc agt	4119
Gly Glu	Val Leu Glu Thr Ser	Thr Ser Ala Val Gly	Asp Leu Ser	
1340	1345	1350		
gga ctt	aat tot gga gga gaa	gtt cta gag att tot	gtc tot gga	4164
Gly Leu	Pro Ser Gly Gly Glu	Val Leu Glu Ile Ser	Val Ser Gly	
1355	1360	1365		
gta gag	gac atc agt ggg ctt	aat tot gga gag gtt	gta gag aat	4209
Val Glu	Asp Ile Ser Gly Leu	Pro Ser Gly Glu Val	Val Glu Thr	
1370	1375	1380		
tot gcc	tot gga ata gag gat	gtc agt gaa ctt aat	taa gga gaa	4254
Ser Ala	Ser Gly Ile Glu Asp	Val Ser Glu Leu Pro	Ser Gly Glu	
1385	1390	1395		
ggt cta	gag acc tot gat tot	gga gta gag gac ctc	agg agg ctc	4299
Gly Leu	Glu Thr Ser Ala Ser	Gly Val Glu Asp Leu	Ser Arg Leu	
1400	1405	1410		
aat tot	gga gaa gaa gtt cta	gag att tot gcc tot	gga ttt ggg	4344
Pro Ser	Gly Glu Glu Val Leu	Glu Ile Ser Ala Ser	Gly Phe Gly	
1415	1420	1425		
gac ctc	agt gga gtt aat tot	gga gga gaa ggt cta	gag acc tot	4389
Asp Leu	Ser Gly Val Pro Ser	Gly Gly Glu Gly Leu	Glu Thr Ser	
1430	1435	1440		
gat tot	gaa gta ggg aat gac	ctc agt ggg ctt aat	tot gga agg	4434
Ala Ser	Glu Val Gly Thr Asp	Leu Ser Gly Leu Pro	Ser Gly Arg	
1445	1450	1455		
gag ggt	cta gag aat taa gat	tot gga gat gag gac	ctc agt gga	4479

Glu Gly	Leu Glu Thr Ser Ala	Ser Gly Ala Glu Asp	Leu Ser Gly	
1460	1465	1470		
ttg cct	tct gga aaa gaa gac	ttg gtg ggg tca gct	tct gga gac	4624
Leu Pro	Ser Gly Lys Glu Asp	Leu Val Gly Ser Ala	Ser Gly Asp	
1475	1480	1485		
ttg gac	ttg ggc aaa ctg cct	tct gga act cta gga	agt ggg caa	4660
Leu Asp	Leu Gly Lys Leu Pro	Ser Gly Thr Leu Gly	Ser Gly Gln	
1490	1495	1500		
gct cca	gaa aca agt ggt ctt	ccc tct gga ttt agt	ggg gag tat	4614
Ala Pro	Glu Thr Ser Gly Leu	Pro Ser Gly Phe Ser	Gly Glu Tyr	
1505	1510	1515		
tct ggg	gtg gac ctt gga agt	ggc cca ccc tct ggc	ctg cct gac	4659
Ser Gly	Val Asp Leu Gly Ser	Gly Pro Pro Ser Gly	Leu Pro Asp	
1520	1525	1530		
ttt agt	gga ctt cca tct gga	ttc cca act gtt tcc	cta gtg gat	4704
Phe Ser	Gly Leu Pro Ser Gly	Phe Pro Thr Val Ser	Leu Val Asp	
1535	1540	1545		
tct aca	ttg gtg gaa gtg gtc	aca gcc tcc act gca	agt gaa ctg	4749
Ser Thr	Leu Val Glu Val Val	Thr Ala Ser Thr Ala	Ser Glu Leu	
1550	1555	1560		
gaa ggg	agg gga acc att ggc	atc agt ggt gca gga	gaa ata tct	4794
Glu Gly	Arg Gly Thr Ile Gly	Ile Ser Gly Ala Gly	Glu Ile Ser	
1565	1570	1575		
gga ctg	ccc tcc agt gag ctg	gac att agt ggg aga	gct agt gga	4839
Gly Leu	Pro Ser Ser Glu Leu	Asp Ile Ser Gly Arg	Ala Ser Gly	
1580	1585	1590		
ctc cct	tca gga act gaa ctc	agt ggc caa gca tct	ggg tct cct	4884

Leu Pro	Ser Gly Thr Glu Leu	Ser Gly Gln Ala Ser	Gly Ser Pro	
1595	1600	1605		
gat gtc	agt ggg gaa ata cct	gga ctc ttt ggt gtc	agt gga cag	4929
Asp Val	Ser Gly Glu Ile Pro	Gly Leu Phe Gly Val	Ser Gly Gln	
1610	1615	1620		
cca tca	ggg ttt cct gac act	agt ggg gaa aca tct	gga gtg act	4974
Pro Ser	Gly Phe Pro Asp Thr	Ser Gly Glu Thr Ser	Gly Val Thr	
1625	1630	1635		
gag att	agg ggg ctg tcc tct	gga caa cca ggt gtt	agt gga gaa	5019
Glu Leu	Ser Gly Leu Ser Ser	Gly Gln Pro Gly Val	Ser Gly Glu	
1640	1645	1650		
gca tct	aga gtt att tat ggc	act agt caa ccc ttt	ggc ata act	5064
Ala Ser	Gly Val Leu Tyr Gly	Thr Ser Gln Pro Phe	Gly Ile Thr	
1655	1660	1665		
gat ctg	agt gga gaa aca tct	ggg gtc cct gat ctc	agt ggg cag	5109
Asp Leu	Ser Gly Glu Thr Ser	Gly Val Pro Asp Leu	Ser Gly Gln	
1670	1675	1680		
cct tca	ggg tta cca ggg ttc	agt ggg gca aca tca	gga gtc cct	5164
Pro Ser	Gly Leu Pro Gly Phe	Ser Gly Ala Thr Ser	Gly Val Pro	
1685	1690	1695		
gac ctg	gtt tct ggt acc acg	agt ggc acc ggt gaa	tct tct ggg	5199
Asp Leu	Val Ser Gly Thr Thr	Ser Gly Ser Gly Glu	Ser Ser Gly	
1700	1705	1710		
att aca	ttt gtg gac acc agt	tig gtt gaa gtg gcc	cct act aca	5244
Ile Thr	Phe Val Asp Thr Ser	Leu Val Glu Val Ala	Pro Thr Thr	
1715	1720	1725		
ttt aca	gaa gaa gaa ggc tta	ggg tct gtg gaa ctc	agt ggc ctc	5289

Phe Lys	Glu Glu Glu Gly Leu	Gly Ser Val Glu Leu	Ser Gly Leu	
1730	1735	1740		
cct tcc	gga gag gca gat ctg	tcg ggc aaa tct ggg	atg gtg gat	5334
Pro Ser	Gly Glu Ala Asp Leu	Ser Gly Lys Ser Gly	Met Val Asp	
1745	1750	1755		
gtc agt	gga cag ttt tct gga	aca gtc gat tcc agt	ggg ttt aca	5379
Val Ser	Gly Gln Phe Ser Gly	Thr Val Asp Ser Ser	Gly Phe Thr	
1760	1765	1770		
tcc cag	act cag gaa ttc agt	ggc cta cca agt ggc	ata gat gag	5424
Ser Gln	Thr Pro Glu Phe Ser	Gly Leu Pro Ser Gly	Ile Ala Glu	
1775	1780	1785		
gtc agt	gga gaa tcc tcc aga	gat gag att ggg agc	agc ctg ccc	5469
Val Ser	Gly Glu Ser Ser Arg	Ala Glu Ile Gly Ser	Ser Leu Pro	
1790	1795	1800		
tcc gga	gca tat tat ggc agt	gga act cca tct agt	ttc ccc acg	5514
Ser Gly	Ala Tyr Tyr Gly Ser	Gly Thr Pro Ser Ser	Phe Pro Thr	
1805	1810	1815		
gtc tct	ctt gta gac aga act	tig gtg gaa tct gta	acc cag gct	5559
Val Ser	Leu Val Asp Arg Thr	Leu Val Glu Ser Val	Thr Gln Ala	
1820	1825	1830		
cca aca	gcc caa gag gca gga	gaa ggg cct tct ggc	att tta gaa	5604
Pro Thr	Ala Gln Glu Ala Gly	Glu Gly Pro Ser Gly	Ile Leu Glu	
1835	1840	1845		
ctc agt	ggg gat cat tct gga	gca cca gac atg tct	ggg gag cat	5649
Leu Ser	Gly Ala His Ser Gly	Ala Pro Asp Met Ser	Gly Glu His	
1850	1855	1860		
tct gga	ttt ctg gac cta agt	ggg ctg cag tcc ggg	ctg ata gag	5694

Ser Gly	Phe	Leu	Asp	Leu	Ser	Gly	Leu	Gln	Ser	Gly	Leu	Ile	Glu	
1865					1870					1875				
ccc agc	gga gag	cca cca	ggt	act	cca tat	ttt agt	ggg gat	ttt		5739				
Pro Ser	Gly Glu	Pro Pro	Gly	Thr	Pro Tyr	Phe Ser	Gly Asp	Phe						
1880				1885			1890							
goc agc	acc acc	aat gta	agt	gga gaa	taa tct	gta	goc atg	ggc		5784				
Ala Ser	Thr Thr	Asn Val	Ser	Gly Glu	Ser Ser	Val	Ala Met	Gly						
1895				1900			1905							
acc agt	gga gag	goc taa	gga	ctt cca	gaa gtt	act	tta atc	act		5829				
Thr Ser	Gly Glu	Ala Ser	Gly	Leu Pro	Glu Val	Thr	Leu Ile	Thr						
1910				1915			1920							
tct gag	tta gtg	gag ggt	gtt	act gaa	cca act	att	tct cag	gaa		5874				
Ser Glu	Phe Val	Glu Gly	Val	Thr Glu	Pro Thr	Ile	Ser Gln	Glu						
1925				1930			1935							
cta ggc	caa agg	ccc cct	gtg	aca cac	aca ccc	cag	ctt ttt	gag		5919				
Leu Gly	Gln Arg	Pro Pro	Val	Thr His	Thr Pro	Gln	Leu Phe	Glu						
1940				1945			1950							
tcg agt	gga aaa	gtc tcc	aca	gct ggg	gac att	agt	gga gct	acc		5964				
Ser Ser	Gly Lys	Val Ser	Thr	Ala Gly	Asp Ile	Ser	Gly Ala	Thr						
1955				1960			1965							
cca gtg	ctc cct	ggg tct	gga	gta gaa	gtg tca	tca	gtc cca	gaa		6009				
Pro Val	Leu Pro	Gly Ser	Gly	Val Glu	Val Ser	Ser	Val Pro	Glu						
1970				1975			1980							
tct agc	agt gag	acc tcc	goc	tat cct	gaa gct	ggg	tta gag	gaa		6054				
Ser Ser	Ser Glu	Thr Ser	Ala	Tyr Pro	Glu Ala	Gly	Phe Gly	Ala						
1985				1990			1995							
tct ggc	goc cct	gag goc	agg	aga gaa	gat tct	ggg	tcc cct	gat		6099				

Ser Ala	Ala Pro	Glu Ala	Ser Arg	Glu Asp	Ser Gly	Ser Pro	Asp	
2000			2005			2010		
ctg agt	gaa acc	acc tct	gca ttc	cac gaa	gct aac	ott gag	aga	6144
Leu Ser	Glu Thr	Thr Ser	Ala Phe	His Glu	Ala Asn	Leu Glu	Arg	
2015			2020			2025		
taa tct	ggc cta	gga gtg	agg ggc	acc act	ttg aca	ttt caa	gaa	6189
Ser Ser	Gly Leu	Gly Val	Ser Gly	Ser Thr	Leu Thr	Phe Gln	Glu	
2030			2035			2040		
ggc gag	ggc tcc	gct gcc	cca gaa	gtg agt	gga gaa	tcc acc	acc	6234
Gly Glu	Ala Ser	Ala Ala	Pro Glu	Val Ser	Gly Glu	Ser Thr	Thr	
2045			2050			2055		
acc agt	gat gtg	ggg aca	gag gca	cca ggc	ttg cct	tca gcc	act	6279
Thr Ser	Asp Val	Gly Thr	Glu Ala	Pro Gly	Leu Pro	Ser Ala	Thr	
2060			2065			2070		
ccc acc	gct tct	gga gac	agg act	gaa atc	agg gga	gac ctg	tct	6324
Pro Thr	Ala Ser	Gly Asp	Arg Thr	Glu Ile	Ser Gly	Asp Leu	Ser	
2075			2080			2085		
ggt cac	acc tgg	cag ctg	gac gtt	gtc atc	agg acc	agg atc	cca	6369
Gly His	Thr Ser	Gln Leu	Gly Val	Val Ile	Ser Thr	Ser Ile	Pro	
2090			2095			2100		
gag tct	gag tag	acc cag	cag acc	cag cgc	cct gca	gag acc	cct	6414
Glu Ser	Glu Trp	Thr Gln	Gln Thr	Gln Arg	Pro Ala	Glu Thr	His	
2105			2110			2115		
cta gaa	att gag	tcc tca	agg ctc	ctg tcc	tca gga	gaa gag	act	6459
Leu Glu	Ile Glu	Ser Ser	Ser Leu	Leu Tyr	Ser Gly	Glu Glu	Thr	
2120			2125			2130		
cac acc	gtc gaa	aca gcc	acc tcc	cca acc	gat gct	tcc atc	cca	6504

His Thr	Val Glu Thr Ala Thr	Ser Pro Thr Asp Ala	Ser Ile Pro	
2135	2140	2145		
gct tct ccg gaa tgg aaa cgt	gaa tca gaa tca act	gct gca gac	6549	
Ala Ser	Pro Glu Trp Lys Arg	Glu Ser Glu Ser Thr	Ala Ala Asp	
2150	2155	2160		
cag gag gta tgt gag gag ggc	tgg aac aag tac cag	ggc cac tgt	6594	
Gln Glu	Val Cys Glu Glu Gly	Trp Asn Lys Tyr Gln	Gly His Cys	
2165	2170	2175		
tac cgc cac ttc ccg gac cgc	gag aac tgg gtg gat	gct gag cgc	6639	
Tyr Arg	His Phe Pro Asp Arg	Glu Thr Trp Val Asp	Ala Glu Arg	
2180	2185	2190		
ccg tgt ccg gag cag cag tca	cac ctg agc agc atc	gtc acc ccc	6684	
Arg Cys	Arg Glu Gln Gln Ser	His Leu Ser Ser Ile	Val Thr Pro	
2195	2200	2205		
gag gag cag gag ttt gtc aac	aac aat gcc caa gac	tac cag tgg	6729	
Glu Glu	Gln Glu Phe Val Asn	Asn Asn Ala Gln Asp	Tyr Gln Trp	
2210	2215	2220		
atc ggc ctg aac gac agg acc	atc gaa gag gac ttc	cgc tgg tca	6774	
Ile Gly	Leu Asn Asp Arg Thr	Ile Glu Gly Asp Phe	Arg Trp Ser	
2225	2230	2235		
gat gga cac ccc atg caa ttt	gag aac tgg cgc ccc	aac cag cct	6819	
Asp Gly	His Pro Met Gln Phe	Glu Asn Trp Arg Pro	Asn Gln Pro	
2240	2245	2250		
gac aac ttt ttt gcc gct gga	gag gac tgt gtg atg	atg etc tgg	6864	
Asp Asn	Phe Phe Ala Ala Gly	Glu Asp Cys Val Val	Met Ile Trp	
2255	2260	2265		
cac gag aag ggc gag tgg aat	gat gtt ccc tgc aat	tac cac ctg	6909	

His Glu Lys Gly Glu Trp Asn Asp Val Pro Cys Asn Tyr His Leu
 2270 2275 2280

ccc ttc acg tgt aaa aag ggc aca gcc acc acc tac aas cgc agc 6954
 Pro Phe Thr Cys Lys Lys Gly Thr Ala Thr Thr Tyr Lys Arg Arg
 2285 2290 2295

cta cag aag cgg agc tca cgg cac cct cgg agc agc ccc agc 6999
 Leu Glu Lys Arg Ser Ser Arg His Pro Arg Arg Ser Arg Pro Ser
 2300 2305 2310

aca gcc cac tga gaagagcttc caggacgcac ccaggacgct gagcccagga 7051
 Thr Ala His
 2315

gcctgccagg ctgacgtgca tcccacccag aggtgtcct ctctctgtcg cttttgtca , 7111

tstaaggast cccattaaaa aaaaaa 7137

<210> 20

<211> 2316

<212> PRT

<213> Homo sapiens

<400> 20

Met Thr Thr Leu Leu Trp Val Phe Val Thr Leu Arg Val Ile Thr Ala
 1 5 10 15

Ala Val Thr Val Glu Thr Ser Asp His Asp Asn Ser Leu Ser Val Ser
 20 25 30

Ile Pro Gln Pro Ser Pro Leu Arg Val Leu Leu Gly Thr Ser Leu Thr

35

40

45

Ile Pro Cys Tyr Phe Ile Asp Pro Met His Pro Val Thr Thr Ala Pro

50

55

60

Ser Thr Ala Pro Leu Ala Pro Arg Ile Lys Trp Ser Arg Val Ser Lys

65

70

75

80

Glu Lys Glu Val Val Leu Leu Val Ala Thr Glu Gly Arg Val Arg Val

85

90

95

Asn Ser Ala Tyr Gln Asp Lys Val Ser Leu Pro Asn Tyr Pro Ala Ile

100

105

110

Pro Ser Asp Ala Thr Leu Glu Val Gln Ser Leu Arg Ser Asn Asp Ser

115

120

125

Gly Val Tyr Arg Cys Glu Val Met His Gly Ile Glu Asp Ser Glu Ala

130

135

140

Thr Leu Glu Val Val Val Lys Gly Ile Val Phe His Tyr Arg Ala Ile

145

150

155

160

Ser Thr Arg Tyr Thr Leu Asp Phe Asp Arg Ala Gln Arg Ala Cys Leu

165

170

175

Gln Asn Ser Ala Ile Ile Ala Thr Pro Glu Gln Leu Gln Ala Ala Tyr

180	185	190
Glu Asp Gly Phe His Gln Cys Asp Ala Gly Trp Leu Ala Asp Gln Thr		
195	200	205
Val Arg Tyr Pro Ile His Thr Pro Arg Glu Gly Cys Tyr Gly Asp Lys		
210	215	220
Asp Glu Phe Pro Gly Val Arg Thr Tyr Gly Ile Arg Asp Thr Asn Glu		
225	230	235 240
Thr Tyr Asp Val Tyr Cys Phe Ala Glu Glu Met Glu Gly Glu Val Phe		
245	250	255
Tyr Ala Thr Ser Pro Glu Lys Phe Thr Phe Gln Glu Ala Ala Asn Glu		
260	265	270
Cys Arg Arg Leu Gly Ala Arg Leu Ala Thr Thr Gly His Val Tyr Leu		
275	280	285
Ala Trp Gln Ala Gly Met Asp Met Cys Ser Ala Gly Trp Leu Ala Asp		
290	295	300
Arg Ser Val Arg Tyr Pro Ile Ser Lys Ala Arg Pro Asn Cys Gly Gly		
305	310	315 320
Asn Leu Leu Gly Val Arg Thr Val Tyr Val His Ala Asn Gln Thr Gly		

325

330

335

Tyr Pro Asp Pro Ser Ser Arg Tyr Asp Ala Ile Cys Tyr Thr Gly Glu

340

345

350

Asp Phe Val Asp Ile Pro Glu Asn Phe Phe Gly Val Gly Gly Glu Glu

355

360

365

Asp Ile Thr Val Gln Thr Val Thr Trp Pro Asp Met Glu Leu Pro Leu

370

375

380

Pro Arg Asn Ile Thr Glu Gly Glu Ala Arg Gly Ser Val Ile Leu Thr

385

390

395

400

Val Lys Pro Ile Phe Glu Val Ser Pro Ser Pro Leu Glu Pro Glu Glu

405

410

415

Pro Phe Thr Phe Ala Pro Glu Ile Gly Ala Thr Ala Phe Ala Glu Val

420

425

430

Glu Asn Glu Thr Gly Glu Ala Thr Arg Pro Trp Gly Phe Pro Thr Pro

435

440

445

Gly Leu Gly Pro Ala Thr Ala Phe Thr Ser Glu Asp Leu Val Val Gln

450

455

460

Val Thr Ala Val Pro Gly Gln Pro His Leu Pro Gly Gly Val Val Phe

465 470 475 480

His Tyr Arg Pro Gly Pro Thr Arg Tyr Ser Leu Thr Phe Glu Glu Ala
485 490 495

Gln Gln Ala Cys Pro Gly Thr Gly Ala Val Ile Ala Ser Pro Glu Gln
500 505 510

Leu Glu Ala Ala Tyr Glu Ala Gly Tyr Glu Glu Cys Asp Ala Gly Trp
515 520 525

Leu Arg Asp Glu Thr Val Arg Tyr Pro Ile Val Ser Pro Arg Thr Pro
530 535 540

Cys Val Gly Asp Lys Asp Ser Ser Pro Gly Val Arg Thr Tyr Gly Val
545 550 555 560

Arg Pro Ser Thr Glu Thr Tyr Asp Val Tyr Cys Phe Val Asp Arg Leu
565 570 575

Glu Gly Glu Val Phe Phe Ala Thr Arg Leu Glu Gln Phe Thr Phe Gln
580 585 590

Glu Ala Leu Glu Phe Cys Glu Ser His Asn Ala Thr Ala Thr Thr Gly
595 600 605

Gln Leu Tyr Ala Ala Trp Ser Arg Gly Leu Asp Lys Cys Tyr Ala Gly

610

615

620

Trp Leu Ala Asp Gly Ser Leu Arg Tyr Pro Ile Val Thr Pro Arg Pro

625

630

635

640

Ala Cys Gly Gly Asp Lys Pro Gly Val Arg Thr Val Tyr Leu Tyr Pro

645

650

655

Asn Gln Thr Gly Leu Pro Asp Pro Leu Ser Arg His His Ala Phe Cys

660

665

670

Phe Arg Gly Ile Ser Ala Val Pro Ser Pro Gly Glu Glu Glu Gly Gly

675

680

685

Thr Pro Thr Ser Pro Ser Gly Val Glu Glu Trp Ile Val Thr Gln Val

690

695

700

Val Pro Gly Val Ala Ala Val Pro Val Glu Glu Glu Thr Thr Ala Val

705

710

715

720

Pro Ser Gly Glu Thr Thr Ala Ile Leu Glu Phe Thr Thr Glu Pro Glu

725

730

735

Asn Gln Thr Glu Trp Glu Pro Ala Tyr Thr Pro Val Gly Thr Ser Pro

740

745

750

Leu Pro Gly Ile Leu Pro Thr Trp Pro Pro Thr Gly Ala Glu Thr Glu

755

760

765

Glu Ser Thr Glu Gly Pro Ser Ala Thr Glu Val Pro Ser Ala Ser Glu

770

775

780

Glu Pro Ser Pro Ser Glu Val Pro Phe Pro Ser Glu Glu Pro Ser Pro

785

790

795

800

Ser Glu Glu Pro Phe Pro Ser Val Arg Pro Phe Pro Ser Val Glu Leu

805

810

815

Phe Pro Ser Glu Glu Pro Phe Pro Ser Lys Glu Pro Ser Pro Ser Glu

820

825

830

Glu Pro Ser Ala Ser Glu Glu Pro Tyr Thr Pro Ser Pro Pro Glu Pro

835

840

845

Ser Trp Thr Glu Leu Pro Ser Ser Gly Glu Glu Ser Gly Ala Pro Asp

850

855

860

Val Ser Gly Asp Phe Thr Gly Ser Gly Asp Val Ser Gly His Leu Asp

865

870

875

880

Phe Ser Gly Gln Leu Ser Gly Asp Arg Ala Ser Gly Leu Pro Ser Gly

885

890

895

Asp Leu Asp Ser Ser Gly Leu Thr Ser Thr Val Gly Ser Gly Leu Thr

900

905

910

Val Glu Ser Gly Leu Pro Ser Gly Asp Glu Glu Arg Ile Glu Trp Pro

915

920

925

Ser Thr Pro Thr Val Gly Glu Leu Pro Ser Gly Ala Glu Ile Leu Glu

930

935

940

Gly Ser Ala Ser Gly Val Gly Asp Leu Ser Gly Leu Pro Ser Gly Glu

945

950

955

960

Val Leu Glu Thr Ser Ala Ser Gly Val Gly Asp Leu Ser Gly Leu Pro

965

970

975

Ser Gly Glu Val Leu Glu Thr Thr Ala Pro Gly Val Glu Asp Ile Ser

980

985

990

Gly Leu Pro Ser Gly Glu Val Leu Glu Thr Thr Ala Pro Gly Val Glu

995

1000

1005

Asp Ile Ser Gly Leu Pro Ser Gly Glu Val Leu Glu Thr Thr Ala

1010

1015

1020

Pro Gly Val Glu Asp Ile Ser Gly Leu Pro Ser Gly Glu Val Leu

1025

1030

1035

Glu Thr Thr Ala Pro Gly Val Glu Asp Ile Ser Gly Leu Pro Ser

1040

1045

1050

Gly Glu Val Leu Glu Thr Thr Ala Pro Gly Val Glu Asp Ile Ser

1055

1060

1065

Gly Leu Pro Ser Gly Glu Val Leu Glu Thr Ala Ala Pro Gly Val

1070

1075

1080

Glu Asp Ile Ser Gly Leu Pro Ser Gly Glu Val Leu Glu Thr Ala

1085

1090

1095

Ala Pro Gly Val Glu Asp Ile Ser Gly Leu Pro Ser Gly Glu Val

1100

1105

1110

Leu Glu Thr Ala Ala Pro Gly Val Glu Asp Ile Ser Gly Leu Pro

1115

1120

1125

Ser Gly Glu Val Leu Glu Thr Ala Ala Pro Gly Val Glu Asp Ile

1130

1135

1140

Ser Gly Leu Pro Ser Gly Glu Val Leu Glu Thr Ala Ala Pro Gly

1145

1150

1155

Val Glu Asp Ile Ser Gly Leu Pro Ser Gly Glu Val Leu Glu Thr

1160

1165

1170

Ala Ala Pro Gly Val Glu Asp Ile Ser Gly Leu Pro Ser Gly Glu

1175

1180

1185

Val Leu Glu Thr Ala Ala Pro Gly Val Glu Asp Ile Ser Gly Leu

1190

1195

1200

Pro Ser Gly Glu Val Leu Glu Thr Ala Ala Pro Gly Val Glu Asp

1205

1210

1215

Ile Ser Gly Leu Pro Ser Gly Glu Val Leu Glu Thr Ala Ala Pro

1220

1225

1230

Gly Val Glu Asp Ile Ser Gly Leu Pro Ser Gly Glu Val Leu Glu

1235

1240

1245

Thr Ala Ala Pro Gly Val Glu Asp Ile Ser Gly Leu Pro Ser Gly

1250

1255

1260

Glu Val Leu Glu Thr Ala Ala Pro Gly Val Glu Asp Ile Ser Gly

1265

1270

1275

Leu Pro Ser Gly Glu Val Leu Glu Thr Thr Ala Pro Gly Val Glu

1280

1285

1290

Glu Ile Ser Gly Leu Pro Ser Gly Glu Val Leu Glu Thr Thr Ala

1295

1300

1305

Pro Gly Val Asp Glu Ile Ser Gly Leu Pro Ser Gly Glu Val Leu

1310

1315

1320

Glu Thr Thr Ala Pro Gly Val Glu Glu Ile Ser Gly Leu Pro Ser

1325

1330

1335

Gly Glu Val Leu Glu Thr Ser Thr Ser Ala Val Gly Asp Leu Ser

1340

1345

1350

Gly Leu Pro Ser Gly Gly Glu Val Leu Glu Ile Ser Val Ser Gly

1355

1360

1365

Val Glu Asp Ile Ser Gly Leu Pro Ser Gly Glu Val Val Glu Thr

1370

1375

1380

Ser Ala Ser Gly Ile Glu Asp Val Ser Glu Leu Pro Ser Gly Glu

1385

1390

1395

Gly Leu Glu Thr Ser Ala Ser Gly Val Glu Asp Leu Ser Arg Leu

1400

1405

1410

Pro Ser Gly Glu Glu Val Leu Glu Ile Ser Ala Ser Gly Phe Gly

1415

1420

1425

Asp Leu Ser Gly Val Pro Ser Gly Gly Glu Gly Leu Glu Thr Ser

1430

1435

1440

Ala Ser Glu Val Gly Thr Asp Leu Ser Gly Leu Pro Ser Gly Arg

1445

1450

1455

Glu Gly Leu Glu Thr Ser Ala Ser Gly Ala Glu Asp Leu Ser Gly

1460

1465

1470

Leu Pro Ser Gly Lys Glu Asp Leu Val Gly Ser Ala Ser Gly Asp

1475

1480

1485

Leu Asp Leu Gly Lys Leu Pro Ser Gly Thr Leu Gly Ser Gly Gln

1490

1495

1500

Ala Pro Glu Thr Ser Gly Leu Pro Ser Gly Phe Ser Gly Glu Tyr

1505

1510

1515

Ser Gly Val Asp Leu Gly Ser Gly Pro Pro Ser Gly Leu Pro Asp

1520

1525

1530

Phe Ser Gly Leu Pro Ser Gly Phe Pro Thr Val Ser Leu Val Asp

1535

1540

1545

Ser Thr Leu Val Glu Val Val Thr Ala Ser Thr Ala Ser Glu Leu

1550

1555

1560

Glu Gly Arg Gly Thr Ile Gly Ile Ser Gly Ala Gly Glu Ile Ser

1565

1570

1575

Gly Leu Pro Ser Ser Glu Leu Asp Ile Ser Gly Arg Ala Ser Gly

1580

1585

1590

Leu Pro Ser Gly Thr Glu Leu Ser Gly Gln Ala Ser Gly Ser Pro

1595

1600

1605

Asp Val Ser Gly Glu Ile Pro Gly Leu Phe Gly Val Ser Gly Gln

1610

1615

1620

Pro Ser Gly Phe Pro Asp Thr Ser Gly Glu Thr Ser Gly Val Thr

1625

1630

1635

Glu Leu Ser Gly Leu Ser Ser Gly Gln Pro Gly Val Ser Gly Glu

1640

1645

1650

Ala Ser Gly Val Leu Tyr Gly Thr Ser Gln Pro Phe Gly Ile Thr

1655

1660

1665

Asp Leu Ser Gly Glu Thr Ser Gly Val Pro Asp Leu Ser Gly Gln

1670

1675

1680

Pro Ser Gly Leu Pro Gly Phe Ser Gly Ala Thr Ser Gly Val Pro

1685

1690

1695

Ala Leu Val Ser Gly Thr Thr Ser Gly Ser Gly Glu Ser Ser Gly

1700

1705

1710

Ile Thr Phe Val Asp Thr Ser Leu Val Glu Val Ala Pro Thr Thr

1715

1720

1725

Phe Lys Glu Glu Glu Gly Leu Gly Ser Val Glu Leu Ser Gly Leu

1730

1735

1740

Pro Ser Gly Glu Ala Asp Leu Ser Gly Lys Ser Gly Met Val Asp

1745

1750

1755

Val Ser Gly Gln Phe Ser Gly Thr Val Asp Ser Ser Gly Phe Thr

1760

1765

1770

Ser Gln Thr Pro Glu Phe Ser Gly Leu Pro Ser Gly Ile Ala Glu

1775

1780

1785

Val Ser Gly Glu Ser Ser Arg Ala Glu Ile Gly Ser Ser Leu Pro

1790

1795

1800

Ser Gly Ala Tyr Tyr Gly Ser Gly Thr Pro Ser Ser Phe Pro Thr

1805

1810

1815

Val Ser Leu Val Asp Arg Thr Leu Val Glu Ser Val Thr Gln Ala

1820

1825

1830

Pro Thr Ala Gln Glu Ala Gly Glu Gly Pro Ser Gly Ile Leu Glu

1835

1840

1845

Leu Ser Gly Ala His Ser Gly Ala Pro Asp Met Ser Gly Glu His

1850

1855

1860

Ser Gly Phe Leu Asp Leu Ser Gly Leu Gln Ser Gly Leu Ile Glu

1865

1870

1875

Pro Ser Gly Glu Pro Pro Gly Thr Pro Tyr Phe Ser Gly Asp Phe

1880

1885

1890

Ala Ser Thr Thr Asn Val Ser Gly Glu Ser Ser Val Ala Met Gly

1895

1900

1905

Thr Ser Gly Glu Ala Ser Gly Leu Pro Glu Val Thr Leu Ile Thr

1910

1915

1920

Ser Glu Phe Val Glu Gly Val Thr Glu Pro Thr Ile Ser Gln Glu

1925

1930

1935

Leu Gly Gln Arg Pro Pro Val Thr His Thr Pro Gln Leu Phe Glu

1940

1945

1950

Ser Ser Gly Lys Val Ser Thr Ala Gly Asp Ile Ser Gly Ala Thr

1955

1960

1965

Pro Val Leu Pro Gly Ser Gly Val Glu Val Ser Ser Val Pro Glu

1970

1975

1980

Ser Ser Ser Glu Thr Ser Ala Tyr Pro Glu Ala Gly Phe Gly Ala

1985	1990	1995
Ser Ala Ala Pro Glu Ala Ser	Arg Glu Asp Ser Gly	Ser Pro Asp
2000	2005	2010
Leu Ser Glu Thr Thr Ser Ala	Phe His Glu Ala Asn	Leu Glu Arg
2015	2020	2025
Ser Ser Gly Leu Gly Val Ser	Gly Ser Thr Leu Thr	Phe Gln Glu
2030	2035	2040
Gly Glu Ala Ser Ala Ala Pro	Glu Val Ser Gly Glu	Ser Thr Thr
2045	2050	2055
Thr Ser Asp Val Gly Thr Glu	Ala Pro Gly Leu Pro	Ser Ala Thr
2060	2065	2070
Pro Thr Ala Ser Gly Asp Arg	Thr Glu Ile Ser Gly	Asp Leu Ser
2075	2080	2085
Gly His Thr Ser Gln Leu Gly	Val Val Ile Ser Thr	Ser Ile Pro
2090	2095	2100
Glu Ser Glu Trp Thr Gln Gln	Thr Gln Arg Pro Ala	Glu Thr His
2105	2110	2115
Leu Glu Ile Glu Ser Ser Ser	Leu Leu Tyr Ser Gly	Glu Glu Thr

2120 2125 2130

His Thr Val Glu Thr Ala Thr Ser Pro Thr Asp Ala Ser Ile Pro
2135 2140 2145

Ala Ser Pro Glu Trp Lys Arg Glu Ser Glu Ser Thr Ala Ala Asp
2150 2155 2160

Gln Glu Val Cys Glu Glu Gly Trp Asn Lys Tyr Gln Gly His Cys
2165 2170 2175

Tyr Arg His Phe Pro Asp Arg Glu Thr Trp Val Asp Ala Glu Arg
2180 2185 2190

Arg Cys Arg Glu Gln Gln Ser His Leu Ser Ser Ile Val Thr Pro
2195 2200 2205

Glu Glu Gln Glu Phe Val Asn Asn Asn Ala Gln Asp Tyr Gln Trp
2210 2215 2220

Ile Gly Leu Asn Asp Arg Thr Ile Glu Gly Asp Phe Arg Trp Ser
2225 2230 2235

Asp Gly His Pro Met Gln Phe Glu Asn Trp Arg Pro Asn Gln Pro
2240 2245 2250

Asp Asn Phe Phe Ala Ala Gly Glu Asp Cys Val Val Met Ile Trp

2255

2260

2265

His Glu Lys Gly Glu Trp Asn Asp Val Pro Cys Asn Tyr His Leu

2270

2275

2280

Pro Phe Thr Cys Lys Lys Gly Thr Ala Thr Thr Tyr Lys Arg Arg

2285

2290

2295

Leu Gln Lys Arg Ser Ser Arg His Pro Arg Arg Ser Arg Pro Ser

2300

2305

2310

Thr Ala His

2315

<210> 21

<211> 1108

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (143)..(1096)

<400> 21

gagtgagiga gagggcagag gaattactca atctgigcca ctactgact tgagcctgct 60

tactcactcc aggaatigcca gaggcact ccttgagcc tcttctctca ctccaggact 120

gccagagga gccatcacc aa atg aag act gct tta att ttg ctc agc att 172

Met Lys Thr Ala Leu Ile Leu Leu Ser Ile

	1	5	10	
ttg gga atg gcc tgt gct ttc tca atg aaa aat ttg cat cga aga gtc				220
Leu Gly Met Ala Cys Ala Phe Ser Met Lys Asn Leu His Arg Arg Val				
	15	20	25	
aaa ata gag gat tct gaa gaa aat ggg gtc ttt aag tac agg cca cga				260
Lys Ile Glu Asp Ser Glu Glu Asn Gly Val Phe Lys Tyr Arg Pro Arg				
	30	35	40	
tat tat ctt tac aag cat gcc tac ttt tat cct cat tta aaa cga ttt				316
Tyr Tyr Leu Tyr Lys His Ala Tyr Phe Tyr Pro His Leu Lys Arg Phe				
	45	50	55	
cca gtt cag gcc agt agt gac tca tcc gaa gaa aat gga gat gac agt				364
Pro Val Gln Gly Ser Ser Asp Ser Ser Glu Glu Asn Gly Asp Asp Ser				
	60	65	70	
tca gaa gag gag gag gaa gaa gag gag act tca aat gaa gaa gaa aac				412
Ser Glu Glu Glu Glu Glu Glu Glu Glu Thr Ser Asn Glu Gly Glu Asn				
	75	80	85	90
aat gaa gaa tcc aat gaa gat gaa gac tct gag gct gag aat acc aca				460
Asn Glu Glu Ser Asn Glu Asp Glu Asp Ser Glu Ala Glu Asn Thr Thr				
	95	100	105	
ctt tct gct aca aca ctg ggc tat gga gag gac gcc acg cct ggc aca				508
Leu Ser Ala Thr Thr Leu Gly Tyr Gly Glu Asp Ala Thr Pro Gly Thr				
	110	115	120	
ggg tat aca ggg tta gct gca atc cag ctt ccc aag aag gct ggg gat				556
Gly Tyr Thr Gly Leu Ala Ala Ile Gln Leu Pro Lys Lys Ala Gly Asp				
	125	130	135	
ata aca aac aaa gct aca aaa gag aag gaa agt gat gaa gaa gaa gag				604
Ile Thr Asn Lys Ala Thr Lys Glu Lys Glu Ser Asp Glu Glu Glu Glu				

140	145	150	
gag gaa gag gaa gag aat gaa aac gaa gaa agc gaa gca gaa gtc gat			652
Glu Glu Glu Glu Gly Asn Glu Asn Glu Glu Ser Glu Ala Glu Val Asp			
155	160	165	170
gaa aac gaa caa ggc ata aac ggc aac agt acc aac agc aca gag gca			700
Glu Asn Glu Gln Gly Ile Asn Gly Thr Ser Thr Asn Ser Thr Glu Ala			
	175	180	185
gaa aac ggc aac ggc agc agc gaa gga gac aat gga gaa gaa ggg gaa			748
Glu Asn Gly Asn Gly Ser Ser Gly Gly Asp Asn Gly Glu Glu Gly Glu			
	190	195	200
gaa gaa agt gtc act gga gcc aat gca gaa ggc acc aca gag acc gga			796
Glu Glu Ser Val Thr Gly Ala Asn Ala Glu Gly Thr Thr Glu Thr Gly			
205	210	215	
ggc cag ggc aag ggc acc tcg aag aca aca acc tct cca aat gat ggg			844
Gly Gln Gly Lys Gly Thr Ser Lys Thr Thr Thr Ser Pro Asn Gly Gly			
220	225	230	
ttt gaa cct aca acc cca cca caa gtc tat aga acc act tcc cca cct			892
Phe Glu Pro Thr Thr Pro Pro Gln Val Tyr Arg Thr Thr Ser Pro Pro			
235	240	245	250
ttt ggg aaa acc acc acc gtt gaa tac gag ggg gag tac gaa tac acg			940
Phe Gly Lys Thr Thr Thr Val Glu Tyr Glu Gly Glu Tyr Glu Tyr Thr			
255	260	265	
ggc gtc aat gaa tac gac aat gga tat gaa atc tat gaa agt gag aac			988
Gly Val Asn Glu Tyr Asp Asn Gly Tyr Glu Ile Tyr Glu Ser Glu Asn			
270	275	280	
ggg gaa cct cgt ggg gac aat tac cga gcc tat gaa gat gag tac agc			1036
Gly Glu Pro Arg Gly Asp Asn Tyr Arg Ala Tyr Glu Asp Glu Tyr Ser			

285

290

295

tac ttt aas gga cas ggc tac gat ggc tat gat ggt cag aat tac tac 1084

Tyr Phe Lys Gly Gln Gly Tyr Asp Gly Tyr Asp Gly Gln Asn Tyr Tyr

300

305

310

cac cac cag tga agctccagcc tg

1108

His His Gln

315

<210> 22

<211> 317

<212> PRT

<213> Homo sapiens

<400> 22

Met Lys Thr Ala Leu His Leu Leu Ser Ile Leu Gly Met Ala Cys Ala

1

5

10

15

Phe Ser Met Lys Asn Leu His Arg Arg Val Lys Ile Glu Asp Ser Glu

20

25

30

Glu Asn Gly Val Phe Lys Tyr Arg Pro Arg Tyr Tyr Leu Tyr Lys His

35

40

45

Ala Tyr Phe Tyr Pro His Leu Lys Arg Phe Pro Val Gln Gly Ser Ser

50

55

60

Asp Ser Ser Glu Glu Asn Gly Asp Asp Ser Ser Glu Glu Glu Glu Glu

65

70

75

80

Glu Glu Glu Thr Ser Asn Glu Gly Glu Asn Asn Glu Glu Ser Asn Glu

85

90

95

Asp Glu Asp Ser Glu Ala Glu Asn Thr Thr Leu Ser Ala Thr Thr Leu

100

105

110

Gly Tyr Gly Glu Asp Ala Thr Pro Gly Thr Gly Tyr Thr Gly Leu Ala

115

120

125

Ala Ile Glu Leu Pro Lys Lys Ala Gly Asp Ile Thr Asn Lys Ala Thr

130

135

140

Lys Glu Lys Glu Ser Asp Glu Glu Glu Glu Glu Glu Glu Gly Asn

145

150

155

160

Glu Asn Glu Glu Ser Glu Ala Glu Val Asp Glu Asn Glu Glu Gly Ile

165

170

175

Asn Gly Thr Ser Thr Asn Ser Thr Glu Ala Glu Asn Gly Asn Gly Ser

180

185

190

Ser Gly Gly Asp Asn Gly Glu Glu Gly Glu Glu Glu Ser Val Thr Gly

195

200

205

Ala Asn Ala Glu Gly Thr Thr Glu Thr Gly Gly Glu Gly Lys Gly Thr

210

215

220

Ser Lys Thr Thr Thr Ser Pro Asn Gly Gly Phe Glu Pro Thr Thr Pro
225 230 235 240

Pro Gln Val Tyr Arg Thr Thr Ser Pro Pro Phe Gly Lys Thr Thr Thr
245 250 255

Val Glu Tyr Glu Gly Glu Tyr Glu Tyr Thr Gly Val Asn Glu Tyr Asp
260 265 270

Asn Gly Tyr Glu Ile Tyr Glu Ser Glu Asn Gly Glu Pro Arg Gly Asp
275 280 285

Asn Tyr Arg Ala Tyr Glu Asp Glu Tyr Ser Tyr Phe Lys Gly Gln Gly
290 295 300

Tyr Asp Gly Tyr Asp Gly Gln Asn Tyr Tyr His His Gln
305 310 315

<210> 23

<211> 498

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (19)..(321)

<400> 23

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Met Arg Ala Leu Thr Leu Leu Ala Leu Leu Ala

1

5

10

ctg gcc gca ctt tgc atc gct ggc cag gca ggt gag aag ccc aga ggt 99

Leu Ala Ala Leu Cys Ile Ala Gly Gln Ala Gly Ala Lys Pro Ser Gly

15

20

25

gca gag tcc agc aaa ggt gca gcc ttt gtg tcc aag cag gag ggc agc 147

Ala Glu Ser Ser Lys Gly Ala Ala Phe Val Ser Lys Gln Glu Gly Ser

30

35

40

gag gta gta aag aga ccc agg cgc tac ctg tat caa tgg ctg gga gcc 195

Glu Val Val Lys Arg Pro Arg Arg Tyr Leu Tyr Gln Trp Leu Gly Ala

45

50

55

cca gtc ccc tac ccg gat ccc ctg gag ccc agg agg gag gta tgt gag 243

Pro Val Pro Tyr Pro Asp Pro Leu Glu Pro Arg Arg Glu Val Cys Glu

60

65

70

75

ctc aat ccg gac tgt gac gag ttg gct gac cac atc ggc ttt cag gag 291

Leu Asn Pro Asp Cys Asp Glu Leu Ala Asp His Ile Gly Phe Gln Glu

80

85

90

gcc tat cgg cgc ttc tac gcc ccg gtc tag ggtgtcgtc tgcgtgcctg 341

Ala Tyr Arg Arg Phe Tyr Gly Pro Val

95

100

gcaggcacc ccagttctgc tccctctcag gcacccttct ttcctcttcc ccttgccctt 401

gccttgacct ccagcccta tggatgtggg gtcccaacca tccagctgc tcccaataa 461

actccagagg aggaatctga aaaaaaaaaa aaaaaaa 498

<210> 24

<211> 100

<212> PRT

<213> Homo sapiens

<400> 24

Met Arg Ala Leu Thr Leu Leu Ala Leu Leu Ala Leu Ala Ala Leu Cys
1 5 10 15

Ile Ala Gly Gln Ala Gly Ala Lys Pro Ser Gly Ala Glu Ser Ser Lys
 20 25 30

Gly Ala Ala Phe Val Ser Lys Gln Glu Gly Ser Glu Val Val Lys Arg
 35 40 45

Pro Arg Arg Tyr Leu Tyr Gln Trp Leu Gly Ala Pro Val Pro Tyr Pro
 50 55 60

Asp Pro Leu Glu Pro Arg Arg Glu Val Cys Glu Leu Asn Pro Asp Cys
65 70 75 80

Asp Glu Leu Ala Asp His Ile Gly Phe Gln Glu Ala Tyr Arg Arg Phe
 85 90 95

Tyr Gly Pro Val
 100

<210> 25

<211> 2383

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (320)..(1825)

<400> 25

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agaacgagtt atttcagct gctgactgga gacgtgcac gctcagatc gagagcatt 120

ccactaagg actgcatca aacacacac cggcagactt caagagctc agactgagga 180

gaaagccttt ccttctgtg ctactgtgc tgcctgtgt ttgaagtc cactccttc 240

atggttttt ctgcacacc agaggacct ttctactgc cgtgttttc ttgggtca 300

ttcagcggct gccagagg atg aga ctc ccc aaa ctc ctc act ttc ttg ctt 352

Met Arg Leu Pro Lys Leu Leu Thr Phe Leu Leu

1

5

10

tag tac ctg gat igg ctg gac ctg gaa ttc atc tgc act gtg ttg ggt 400

Trp Tyr Leu Ala Trp Leu Asp Leu Glu Phe Ile Cys Thr Val Leu Gly

15

20

25

gac cct gac ttg gcc cag aga ccc cag ggg acc agc cca gga ttg gcc 448

Ala Pro Asp Leu Gly Gln Arg Pro Gln Gly Thr Arg Pro Gly Leu Ala

30

35

40

aaa gca gag gcc aag gag agc ccc ccc ctg gcc cgg aac gtc ttc agc 496

Lys Ala Glu Ala Lys Glu Arg Pro Pro Leu Ala Arg Asn Val Phe Arg

45

50

55

cca ggg ggt cag agc tat ggt ggg ggc acc aat gcc aat gcc agg 544
 Pro Gly Gly His Ser Tyr Gly Gly Gly Ala Thr Asn Ala Asn Ala Arg
 60 65 70 75

cca aag gga ggc acc ggg cag aca gga ggc ctg aca cag ccc aag aag 592
 Ala Lys Gly Gly Thr Gly Gln Thr Gly Gly Leu Thr Gln Pro Lys Lys
 80 85 90

gat gaa ccc aaa aag ctg ccc ccc aga ccg ggc ggc cct gaa ccc aag 640
 Asp Glu Pro Lys Lys Leu Pro Pro Arg Pro Gly Gly Pro Glu Pro Lys
 95 100 105

cca gga cac cct ccc caa aca ggg cag gct aca gcc cgg act gtg acc 688
 Pro Gly His Pro Pro Gln Thr Arg Gln Ala Thr Ala Arg Thr Val Thr
 110 115 120

cca aaa gga cag ctt ccc gga ggc aag gca ccc cca aaa gca gga tct 726
 Pro Lys Gly Gln Leu Pro Gly Gly Lys Ala Pro Pro Lys Ala Gly Ser
 125 130 135

gtc ccc agc tcc ttc ctg ctg aag aag gcc agg gag ccc ggg ccc cca 784
 Val Pro Ser Ser Phe Leu Leu Lys Lys Ala Arg Glu Pro Gly Pro Pro
 140 145 150 155

cca gag ccc aag gag ccg ttt cgc cca ccc ccc atc aca ccc cac gag 832
 Arg Glu Pro Lys Glu Pro Phe Arg Pro Pro Pro Ile Thr Pro His Glu
 160 165 170

tac atg ctc tgg ctg tac agg acg ctg tcc gat gct gac aga aag gga 880
 Tyr Met Leu Ser Leu Tyr Arg Thr Leu Ser Asp Ala Asp Arg Lys Gly
 175 180 185

ggc aac agc agc gtc aag ttg gag gct ggc ctg gcc aac acc atc acc 928
 Gly Asn Ser Ser Val Lys Leu Glu Ala Gly Leu Ala Asn Thr Ile Thr
 190 195 200

agc ttt att gac aac gag caa gat gac cga ggt ccc gtg gtc agg aag 976
 Ser Phe Ile Asp Lys Gly Gln Asp Asp Arg Gly Pro Val Val Arg Lys
 205 210 215

cag agg tac gtg ttt gac att agt gcc ctg gag aag gat ggg ctg ctg 1024
 Gln Arg Tyr Val Phe Asp Ile Ser Ala Leu Glu Lys Asp Gly Leu Leu
 220 225 230 235

ggg gcc gag ctg cgg atc ttg cgg aag aag ccc tgg gac acg gcc aag 1072
 Gly Ala Glu Leu Arg Ile Leu Arg Lys Lys Pro Ser Asp Thr Ala Lys
 240 245 250

cca ggg gcc ccc gga ggc ggg cgg gct gcc cag ctg aag ctg tcc agc 1120
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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/JP2004/011401

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N5/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-internal, WPI Data, PAJ, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>KALE S ET AL: "THREE-DIMENSIONAL CELLULAR DEVELOPMENT IS ESSENTIAL FOR EX VIVO FORMATION OF HUMAN BONE" NATURE BIOTECHNOLOGY, NATURE PUBLISHING, US, vol. 18, September 2000 (2000-09), pages 954-958, XP000996174 ISSN: 1087-0156</p> <p>the whole document</p>	<p>1-30, 32-34, 42-44, 46,51, 54,55, 58-60, 62-64, 68-85, 88, 92-96, 149-160</p>



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

Date of the actual completion of the international search

29 December 2004

Date of making of the international search report

05/01/2005

Name and mailing address of the ISA

European Patent Office, P.O. Box 5515 Patentplan 2
84, - 2200 HV Rijswijk
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Fax: (+31-70) 340-0010

Authorized officer

Chavasne, F

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/JP2004/011401

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim(s)
X	<p>WO 95/30742 A (BIOSURFACE TECH INC) 16 November 1995 (1995-11-16)</p> <p>abstract page 28 - page 29 page 30, line 12 - page 31, line 7 page 32, line 28 - page 33, line 31 page 36, line 30 - page 40 examples 3,4</p>	<p>1-30, 32-34, 42-48, 51-60, 62-86, 88-112, 114-141, 149-160</p>
X	<p>KUSHIDA A, ET AL.: JOURNAL OF BIOMEDICAL MATERIALS RESEARCH, vol. 45, no. 5, 1999, pages 355-362, XP001204072</p> <p>abstract page 355, column 2 - page 356, column 1, paragraph 1 page 356, column 2, paragraph 2 page 357, column 2, paragraph 2 figure 1 page 359 page 360, column 1, paragraph 2 page 360, column 2, paragraph 2 - page 361, column 1, paragraph 1</p>	<p>1,3-12, 14-23, 26-30, 34-36, 42,43, 48,49, 51-53, 62-64, 74-79, 81-85</p>
X	<p>WO 95/33821 A (ADVANCED TISSUE SCIENCES INC) 14 December 1995 (1995-12-14)</p> <p>abstract page 15, paragraph 2 - page 17, paragraph 1 page 18, paragraph 7 page 19, paragraph 2 - page 30, paragraph 2 page 45, paragraph 1 - page 46, paragraph 1</p>	<p>1-16, 18-30, 32-41, 43-86, 88-112, 114-160</p>

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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/JP2004/011401

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2003/091979 A1 (ESCHENHAGEN THOMAS) 15 May 2003 (2003-05-15) abstract paragraphs '0001!', '0008!', '0014!', '0016!', '0031!', '0083!' ~ '0085!' example 2 -----	1-6, 16, 18-31, 74-77, 85-87, 91-94, 96, 149, 150, 154, 156, 157

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PCT/JP2004/011401

Patent document cited in search report		Publication date		Patent family member(s)		Publication date
WO 9530742	A	16-11-1995	AU	2435495 A		29-11-1995
			AU	2468995 A		29-11-1995
			WO	9530383 A1		16-11-1995
			WO	9530742 A1		16-11-1995
			US	5723331 A		03-03-1998
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2004/021401

Box No. 1 Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
 - a. type of material
 - ☒ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material
 - ☒ in written format
 - ☒ in computer readable form
 - c. time of filing/furnishing
 - ☒ contained in the international application as filed
 - ☒ filed together with the international application in computer readable form
 - ☐ furnished subsequently to this Authority for the purpose of search
2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2004/011401

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 97-141 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.